PERIODICAL
Marine Biological Association of the United Kingdom.
Journal
V. 38
1959

DATE OF MAGAZINE	BORROWER'S NAME	TIME
2/14/64		

PERIODICAL

MArine Biological Association of the United Kingdom.
Journal

V. 38

1959

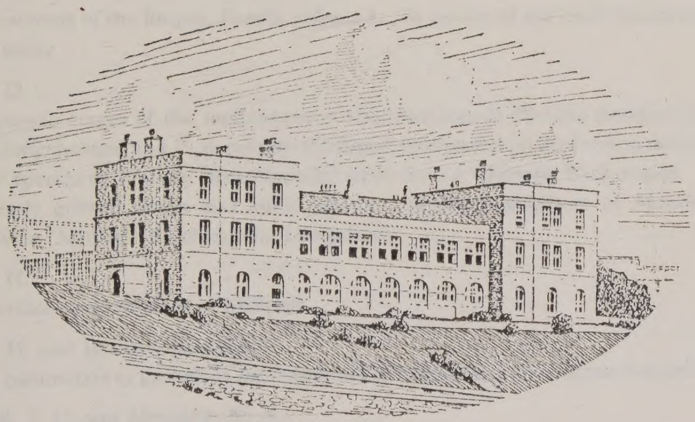
LIBRARY
BUR. OF COMM. FISH.
HON. BIOL. LAB.

2958 6

PERIODICAL

J. of 'Marine
Biol. Assn. of
U. K.
v. 38
1959

JOURNAL OF THE
MARINE BIOLOGICAL ASSOCIATION
OF THE UNITED KINGDOM



THE PLYMOUTH LABORATORY

VOLUME 38

1959

CAMBRIDGE
AT THE UNIVERSITY PRESS
1959

Digitized by the Internet Archive
in 2025

https://archive.org/details/marine-biological-association-uk-journal_1959_38

CONTENTS OF VOLUME 38

Number 1, pp. 1–224, February 1959
Number 2, pp. 225–428, June 1959
Number 3, pp. 429–660, December 1959

PAGE

Report of the Council: 1958–59 633

Income and Expenditure Account and Balance Sheet: 1958–59. 658

Obituary: Dr G. A. Steven, F.R.S.E. 1

Allen, J. A.
On the biology of *Pandalus borealis* Krøyer, with reference to a population off the Northumberland coast 189

Armstrong, F. A. J. and Butler, E. I.
Chemical changes in sea water off Plymouth in 1957 41

Arnold, D. C.
The reactions of the limpet, *Patella vulgata* L., to certain of the ionic constituents of sea water 596

Atkins, D.
The growth stages of the lophophore of the brachiopods *Platidia davidsoni* (Eudes Deslongchamps) and *P. anomioides* (Philippi), with notes on the feeding mechanism 103
A new species of *Platidia* (Brachiopoda) from the La Chapelle Bank region . . 133
The early growth stages and adult structure of the lophophore of *Macandrevia cranium* (Müller) (Brachiopoda, Dallinidae) 335

Barnes, H.
Sea surface temperatures at Millport 423

Barnes, H. and Barnes, Margaret
Some parameters of growth in the common intertidal barnacle, *Balanus balanoides* (L) 581

Barry, R. J. C. and Munday, K. A.
Carbohydrate levels in *Patella* 81

Baxter, I. G., *see* Parrish, *et al.* 445

Boney, A. D. and Corner, E. D. S.
Application of toxic agents in the study of the ecological resistance of intertidal red algae 267

Butler, E. I., *see* Armstrong and Butler 41

Cameron, Jane
The larval and post-larval stages of *Gymnammodytes semisquamatus* (Jourdain) . 17

Carlisle, D. B.
Moulting hormones in *Palaemon* (= *Leander*) (Crustacea Decapoda) II. Differences between populations 351

PAGE

On the sexual biology of *Pandalus borealis* (Crustacea Decapoda)
I. Histology of incretory elements 381
II. The termination of the male phase 481
III. The initiation of the female phase 493

CARLISLE, D. B. and DENTON, E. J.
On the metamorphosis of the visual pigments of *Anguilla anguilla* (L.) 97

CLARKE, B. *see* PARKE, MANTON and CLARKE 209

CORNER, E. D. S., *see* BONEY and CORNER 267

CRAIG, R. E. *see* PARRISH, *et al.* 445

CRISP, D. J. and SOUTHWARD, A. J.
The further spread of *Elminius modestus* in the British Isles to 1959 429

DENTON, E. T., *see* CARLISLE and DENTON 97

DROOP, M. R.
A note on some physical conditions for cultivating *Oxyrrhis marina* 599
Water-soluble factors in the nutrition of *Oxyrrhis marina* 605

EVANS, FRANK
Wind currents, tidal streams and plankton off the Northumberland coast . . . 529

FISHER, L. R. and GOLDIE, E. H.
The food of *Meganyctiphanes norvegica* (M. Sars), with an assessment of the contributions of its components to the vitamin A reserves of the animal 291

FORSTER, G. R.
The ecology of *Echinus esculentus* L. Quantitative distribution and rate of feeding . 361
The biology of the prawn, *Palaemon* (=*Leander*) *serratus* (Pennant) 621

GILPIN-BROWN, J. B.
The reproduction and larval development of *Nereis fucata* (Savigny) 65

GOLDIE, E. H., *see* FISHER and GOLDIE 291

HOLME, N. A.
A hopper for use when sieving bottom samples at sea 525
The British species of *Lutraria* (Lamellibranchia), with a description of *L. angustior* Philippi 557

HOWIE, D. I. D.
The spawning of *Arenicola marina* (L.) I. The breeding season 395

JONES, W. EIFION
The growth and fruiting of *Gracilaria verrucosa* (Hudson) Papenfuss 47
Experiments on some effects of certain environmental factors on *Gracilaria verrucosa* (Hudson) Papenfuss 153

KABATA, Z.
Ecology of the genus *Acanthochondria* Oakley (Copepoda parasitica) 249

KENNEDY, G. Y.
A porphyrin pigment in the integument of *Arion ater* (L.) 27

PAGE

KRISHNASWAMY, S.
Respiration in *Chondracanthus zei* de la Roche (Copepoda) 263
A new species of copepod from the Eddystone shell gravel 543

LLEWELLYN, J.
The larval development of two species of gastrocotylid trematode parasites from the gills of *Trachurus trachurus* 461

MANTON, IRENE
Electron microscopical observations on a very small flagellate: the problem of *Chromulina pusilla* Butcher 319

see also PARKE, MANTON and CLARKE 169

MASON, JAMES
The biology of *Nicothoë astaci* Audouin and Milne Edwards 3

MORTON, J. E.
The habits and feeding organs of *Dentalium entalis* 225

MUNDAY, R. J. C., *see* BARRY and MUNDAY 81

NICOL, J. A. C.
Digestion in sea anemones 469
Studies on luminescence. Attraction of animals to a weak light 477

PARKE, MARY, MANTON, IRENE, and CLARKE, B.
Studies on marine flagellates. V. Morphology and microanatomy of *Chrysochromulina strobilus* sp.nov. 169

PARRISH, B. B., SAVILLE, A., DRAIG, R. E., BAXTER, I. G. and PRIESTLEY, R.
Observations on herring spawning and larval distribution in the Firth of Clyde in 1958 445

PATEL, BHUPENDRA
The influence of temperature on the reproduction and moulting of *Lepas anatifera* L. under laboratory conditions 589

PRIESTLEY, R. *see* PARRISH *et al.* 445

RALPH, P. M.
Notes on the species of the pteromedusan genus *Tetraplatia* Busch, 1851 . . 369

RUSSELL, F. S.
Some observations on the scyphomedusa *Atolla* 33

SAVILLE, A., *see* PARRISH *et al.* 445

SLINN, D. J. *see* THOMPSON and SLINN 507

SOUTHWARD, A. J. *see* CRISP and SOUTHWARD 429

SOUTHWARD, EVE C.
Two new species of Pogonophora from the north-east Atlantic 439

SPOONER, G. M.
The occurrence of *Microcharon* in the Plymouth offshore bottom fauna, with description of a new species 57

STRIDE, A. H.
A linear pattern on the sea floor and its interpretation 313

PAGE

THOMPSON, T. E.
Feeding in nudibranch larvae 239

THOMPSON, T. E. and SLINN, D. J. . . . .
On the biology of the opisthobranch *Pleurobranchus membranaceus*. 507

WIERSMA, C. A. G.
Movement receptors in decapod Crustacea 143

WILSON, DOUGLAS P.
The polychaete *Magelona filiformis* sp.nov. and notes on other species of *Magelona* 547

WOODHEAD, A. D.
Variations in the activity of the thyroid gland of the cod, *Gadus callarias* L., in relation to its migrations in the Barents Sea
I. Seasonal changes 407
II. The 'dummy run' of the immature fish 417

YONGE, C. M.
On the structure, biology and systematic position of *Pharus legumen* (L.) . . 277

YOSHIDA, M.
Naphthaquinone pigments in *Psammechinus miliaris* (Gmelin) 455

ABSTRACTS OF MEMOIRS
RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

On the basic form and adaptations to habitat in the Lucinacea (Eulamellibranchia). By J. A. Allen 221

A new species and genus of Kraussinidae (Brachiopoda) with a note on feeding. By D. Atkins 425

The direct estimation of ammonia in sea water, with notes on nitrate, copper, zinc, and sugars. By W. R. G. Atkins 212

Cube photometer measurements of the angular distribution of submarine daylight and the total submarine illumination. By W. R. G. Atkins and H. H. Poole . . . 221

Evidence for an absorptive function in the 'liver' of *Octopus vulgaris* Lam. By A. M. Bidder 222

Lumière, bioluminescence et migrations de la couche diffusante profonde en Méditerranée occidentale. By B. P. Boden and E. M. Kampa 222

Synaptic relations in the atrial nervous system of amphioxus. By Q. Bone . . 425

Chitin in Pogonophora. By P. C. J. Brunet and D. B. Carlisle 425

Studies on the internal pH of large muscle and nerve fibres. by P. C. Caldwell . 222

Responses of the anterior retractor muscle of the byssus of *Mytilus edulis*. By G. W. Cambridge 223

A crustacean chromactivator. By D. B. Carlisle 223

Consumption of nutrient salts in the English Channel as a means of measuring production. By L. H. N. Cooper 233

PAGE

The contributions of the orientated photosensitive and other molecules to the absorption of whole retina. By E. J. Denton 629

Dactylopusioides macrolabris (Claus) (Copepoda; Harpacticoida) and its frond-mining nauplius. By J. Green 223

A contribution to the biology and cytology of *Haliphysema* (Foraminifera). By R. H. Hedley 224

The initial heat production of stimulated nerve. By A. V. Hill and J. V. Howarth . 425

The effect of reversing the internal water-current of the spicule orientation in *Leucosolenia variabilis* and *L. complicata*. By W. Clifford Jones 224

The response of the ampullae of Lorenzini to combined stimulation by temperature change and weak direct currents. By R. W. Murray 629

Changes in the Chalk heart-urchin *Micraster* interpreted in relation to living forms. By D. Nichols 629

The inorganic and amino-acid composition of some lamellibranch muscles. By W. T. W. Potts 426

Pogonophora from the Altantic. By A. J. Southward and E. C. Southward . . . 224

Abundance of Pogonophora. By A. J. Southward 224

New members of the British marine bottom fauna. By G. M. Spooner 630

BOOK REVIEWS

Oceanography and Marine Biology: A book of Techniques. By H. Barnes 427

Marine Ecology. By Hilary B. Moore 427

Molluscs. By J. E. Morton 428

The Underwater Guide to Marine Life. By Carleton Ray and Elgin Ciampi . . . 428

Endocrine Control in Crustaceans. By D. B. Carlisle and Sir Francis Knowles . 631

G. A. Steven

(*Frontispiece*)

J. mar. biol. Ass. U.K. (1959) 38, 1–2
Printed in Great Britain

OBITUARY

Dr G. A. Steven, F.R.S.E.

George Alexander Steven was born at Freshwick, Caithness, on 13 April 1901. His boyhood was spent among crofter fisherfolk, and fishing and boats were part of his youthful environment. During 1924–28 he studied Zoology in the University of Edinburgh, being Vans Dunlop Scholar in 1926. After graduation he came to Plymouth, first as student probationer and then, a year later in 1929, he was appointed to the permanent staff of the laboratory. In 1931 he was elected a Fellow of the Royal Society of Edinburgh, and in 1952 was awarded the Doctor of Science degree of the University of Edinburgh. After a long illness he died on 7 April 1958 at his home in Yelverton, Devon, leaving a widow and two sons.

The bulk of Steven's scientific work at Plymouth was closely concerned with the commercial fisheries of Devon and Cornwall, but many of his findings have wider application, both in the field of biology and of economics. The work is grouped around the following four major subjects: (1) rays and skates, (2) shags and cormorants, (3) seals, (4) mackerel. He had early realized the importance in the south-west of the ray and skate fishery and the prevailing general ignorance concerning the biology of the fishes on which it is based. His researches established that rays tend to congregate in unispecific and unisexual shoals, that they are slow-growing and that, at any rate in the Thornback Ray, juveniles stay for years on the same ground with little tendency to move away. A result of this habit is the depletion of stocks in areas of intense trawling unless care be taken to return all young rays to the water at once. Investigations into the food of shags and cormorants followed a request from the Cornwall Sea Fisheries Committee. In the belief that these birds menaced the inshore fisheries, especially flatfishes, the Committee had paid away hundreds of pounds for thousands of their heads. Steven was able to show that shags, by far the most numerous of the two birds, eat largely non-marketable fishes and flatfishes scarcely at all. The latter do form an appreciable element in the food of cormorants but these birds are too few to have any marked effect on the fishery. His studies of the seal population of Cornwall similarly arose from complaints of fishermen that seals gravely menaced their livelihood. While making his census Steven visited, often by swimming into them under uncomfortable and risky circumstances, all the caves along the north Cornish coast. He concluded that there were (in 1935) between 300 and 500 Grey Seals in the area. Steven's work on the biology of the mackerel undoubtedly ranks as his major contribution to fishery science.

Through a fog of earlier pre-conceived ideas and conflicting statements he made his way to a rational interpretation of the annual movements of the mackerel shoals, showing that they winter in dense congregations near banks and gulleys on the bottom, rising in the spring to the surface to migrate to a common spawning ground near the 100-fathom contour, which in the western region lies west of the Isles of Scilly. After spawning they migrate into shallow water inshore during the summer, later returning to winter quarters. He also made important observations on spawning, feeding, age and growth.

Steven's scientific work at Plymouth suffered interruption during the war years. He had served in the Army in France during the 1914–18 war, and during the first years of the Second World War he saw active service with the Royal Navy. During 1942–45 he was seconded from the Navy to develop the fisheries of Sierra Leone under the Colonial Office. From his base in Freetown, despite many difficulties and much discouragement, he played a leading part in preparing the groundwork for the post-war fisheries of that region, in so doing producing two comprehensive Reports for the Colony of Sierra Leone.

Just prior to the outbreak of war in 1939 he had accepted the post of Director of the Newfoundland Fisheries Research Laboratory at St Johns. Within a few days a disastrous fire destroyed the St Johns Laboratory and the war intervening before other arrangements could be made he never took up this post.

The research vessel 'Sarsia', the finest ever owned by the Association, owes much in her design and equipment to Steven's practical knowledge of work at sea. He was much interested in net-making, and a useful small handbook he wrote on the subject has been very favourably received.

From 1930 to 1948, exclusive of the war years, Steven took an active part in the Courses in Marine Biology for university students, held at the Plymouth Laboratory each year during the Easter Vacation. There are many zoologists who to-day remember with gratitude his care for them while on their first dredging and trawling expeditions, and the excellence and stimulating manner of his teaching. His many friends and colleagues at the Laboratory and elsewhere will sorely miss him.

D.P.WILSON

J. mar. biol. Ass. U.K. (1959) 38, 3–16
Printed in Great Britain

THE BIOLOGY OF *NICOTHOË ASTACI* AUDOUIN AND MILNE EDWARDS

By James Mason
Marine Laboratory, Aberdeen

(Text-figs. 1–4)

Nicothoë astaci is a choniostomatid copepod (Gurney, 1929) parasitic on the gills of the lobster, *Homarus vulgaris* Milne Edwards. In view of the possible effect of *Nicothoë* on the lobster, which is of considerable commercial importance in Scotland, a programme of work was instituted on the biology of the parasite.

Nicothoë attaches itself by means of its suctorial mouth to a gill filament of the lobster, and the wall of the filament is pierced by the sharp, styliform mandibles. The host's blood is sucked by means of a muscular gullet leading to the stomach. *Nicothoë* often occurs in groups, especially near the bases of the gills. The adult parasite is incapable of any movement except a slight lateral displacement of the abdomen and, once attached, probably remains in the same position throughout its life. Little is known of the life cycle, and all individuals so far definitely recognized have been females.

MATERIAL AND METHODS

Specimens of *Nicothoë astaci* were collected from six samples of preserved Orkney lobsters between September 1954 and November 1955, and, in order more adequately to cover the annual cycle of *Nicothoë*, nine further samples were obtained from Bernera, Lewis, between October 1955 and November 1956. Lobsters arriving in poor condition at two merchants' premises were preserved in formalin and sent to the Marine Laboratory, Aberdeen, for examination. Living parasites were also examined, and stained sections and whole mounts prepared.

THE ADULT FEMALE

The adult female (Figs. 1 and 2) is made up of three parts: (i) cephalothorax; (ii) thorax, which bears two large lateral expansions or wings; and (iii) abdomen, which carries two oval egg-sacs. The length to the tip of the abdomen is some 1·2 to 1·7 mm; each wing, measured from its tip to the junction of its anterior edge with the trunk, is up to some 4 mm long; the egg-sacs measure up to 3 mm.

The segmentation is set out in Table 1 and shown in Fig. 2. The appendages are not figured in detail as this has been done by previous workers (Leigh-Sharpe, 1926; Gurney, 1929).

The first two thoracic segments are fused to the head, and the next three, which are distinguishable dorsally by three rings, are concerned in the formation of the wings. Articulation is between the posterior edge of the wings and the sixth thoracic segment, the latter being fused to the first abdominal segment.

The adult female of *Nicothoë* has the full complement of segments and limbs, and is to be regarded as a modification of, rather than a degeneration from, the typical copepod form.

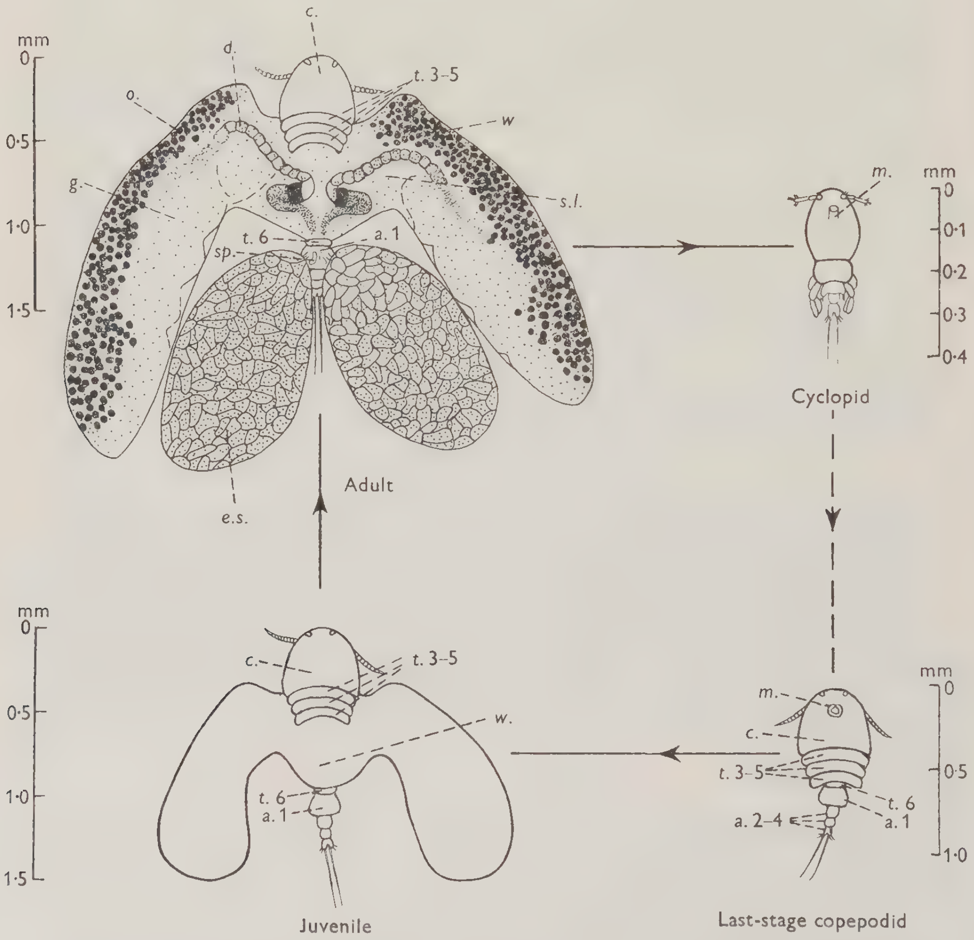

mm
0
0·5
1·0
1·5
c.
d.
o.
t. 3-5
w
g.
s.l.
t. 6
a. 1
sp.
e.s.
Adult
m.
mm
0
0·1
0·2
0·3
0·4
Cyclopid
mm
0
0·5
1·0
1·5
c.
t. 3-5
w.
t. 6
a. 1
Juvenile
m.
c.
t. 3-5
a. 2-4
t. 6
a. 1
mm
0
0·5
1·0
Last-stage copepodid

Fig. 1. Dorsal views of stages in the life cycle of *Nicothoë*. *a.* 1–4, abdominal segments; *c.*, cephalothorax; *d.*, duct; *e.s.*, egg-sac; *g.*, cement gland; *m.*, mouth showing through from ventral surface; *o.*, ovary; *s.l.*, stomach lobe; *sp.*, spermathecae; *t.* 3–6, thoracic segments; *w.*, wings.

TABLE 1. SEGMENTATION IN *NICOTHOË*

Body region	Segment	Appendages: Name	Appendages: Form
Cephalothorax	1. 1st cephalic	—	—
	2. 2nd cephalic	1st antennae	Long, 11-jointed, setigerous
	3. 3rd cephalic	2nd antennae	4-jointed, flexed on themselves
	4. 4th cephalic	Mandibles	Styliform, passing into mouth tube
	5. 5th cephalic	1st maxillae	2-jointed, anterior joint tripartite
	6. 6th cephalic	2nd maxillae	2-jointed, with a pair of lappets distally
	7. 1st thoracic	Maxillipeds	4-jointed, terminal joint small
	8. 2nd thoracic	Biramous limbs	Basal joint and two 3-jointed rami, setigerous
Thorax (wings)	9. 3rd thoracic (dorsal ring)	Biramous limbs	Basal joint and two 3-jointed rami, setigerous
	10. 4th thoracic (dorsal ring)	Biramous limbs	Basal joint and two 3-jointed rami, setigerous
	11. 5th thoracic (dorsal ring)	Biramous limbs	Basal joint and two 3-jointed rami, setigerous
Abdomen	12. 6th thoracic	Uniramous limbs	Vestigial, setigerous
	13. 1st abdominal. Genital	(Egg-sacs)	
	14. 2nd abdominal		
	15. 3rd abdominal		
	16. 4th abdominal. Bilobed	(Each lobe has a caudal style and 4 setae)	

THE BREEDING CYCLE

The paired ovaries are situated one in each wing, where they cover the stomach lobes anteriorly (Fig. 1). Posteriorly each lobe is covered by a cement gland. A dorsal genital duct, which serves both ovary and cement gland, passes proximally in each wing to open on the 1st abdominal segment. The contents of the ducts, thought by Van Beneden (1850) to be eggs, are, in fact, glandular in nature. Near the looped bases of the ducts are dark bodies, thought to be concerned in the secretion of the egg-sacs. In the 1st abdominal segment, near the openings of the oviducts, is a pair of curved spermathecae.

Individuals of *Nicothoë* with small wings but without egg-sacs (juveniles, Fig. 1) are found almost entirely on lobsters which have recently cast their shells (see p. 13). As the wings grow the ovaries begin to develop; they first appear when the wings are some 1·5 mm long, and soon become ripe, generally when the wings have attained a length of 2·0–2·2 mm. The eggs are extruded with the secretion of the duct and cement glands, to form the egg-sacs. The parasites have then become adults, and have empty gonads and egg-sacs full of eggs. Development of the eggs into embryos commences at once, fertilization by sperm from the spermathecae (see p. 11), presumably having occurred before they entered the egg-sacs. The ovaries recover at the same time, and concurrent series of changes take place in the ovaries and egg-sacs, so that when the egg-sacs are full of active larvae ready to be released, the gonads are again full of ripe eggs. The egg-sacs burst, releasing the larvae, the gonad spawns and new egg-sacs are extruded, and the cycle is repeated.

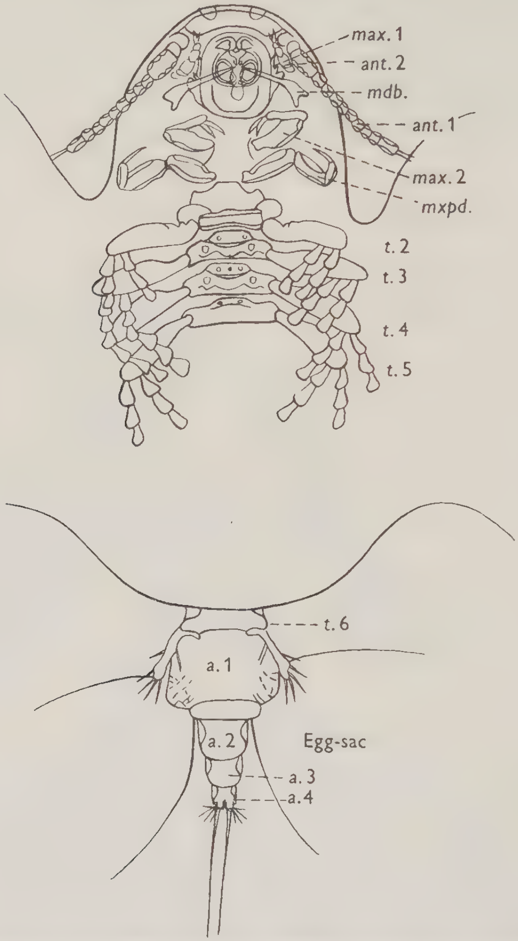


Fig. 2. Ventral view of adult female, showing segmentation (simplified for clarity). *a.* 1–4, abdominal segments; *ant.* 1, 1st antenna; *ant.* 2, 2nd antenna; *max.* 1, 1st maxilla; *max.* 2, 2nd maxilla; *mdb.*, mandible; *mxpd.*, maxilliped; *t.* 2–6, thoracic segments and appendages.

The series of changes in the reproductive system of the juvenile and adult *Nicothoë*, and the corresponding changes in the egg-sacs of the adult, have been divided into six stages, as follows.

Immature (juvenile) or *Spent* (adult). Stomach lobes occupy almost whole of wings. No sign of development of ovaries visible externally in juvenile; adult ovary appears empty or contains a few residual eggs; sections show many young oocytes, 10–12 μ, each with a spherical, vesicular nucleus. Cement glands small, appear translucent externally; contain a little granular secretion, staining pale blue in haematoxylin and eosin, from glandular cells near outer wall of gland. Ducts, 75 μ wide, tapering slightly distally, appear empty, or contents sparse, but sections show that they are composed of a glandular tissue similar to that of cement glands and contain a little granular secretion. Egg-sacs contain spherical eggs, 120–150 μ, or eggs which have just commenced division.

Developing. Ovaries become visible externally as granular, greyish bodies, but with no definite oocytes visible; sections show strings of young oocytes, 10–12 μ, with a few larger ones, up to 30 μ, between. A group of actively dividing oogonia on anterior edge of each wing. Cement glands larger, pale, containing more granular secretion. Glandular tissue in ducts assumes a segmented appearance, being divided up into well-defined packets, some 80 μ long, which also contain granular secretion. Larvae in egg-sacs 150–200 μ.

Half-full. Ovaries darker, definite rounded oocytes up to 75 μ visible externally; strings of young oocytes and groups of actively dividing oogonia seen in sections. Cement glands and ducts appear darker and have similar granular contents, staining pink in haematoxylin and eosin; at centre of cement gland granules are coalescing to form small droplets of a clear fluid which stains pale pink. Larvae in egg-sacs 200–230 μ, showing signs of segmentation.

Full. Ovaries large, full of spherical oocytes up to 95 μ in diameter, each with germinal vesicle 17 μ and nucleolus 4 μ; few young oocytes; patches of oogonia present. Cement glands large, appear opaque externally, but sections show further coalescence of granules to form clear fluid which stains pale pink. Ducts darker, contents granular. Larvae in egg-sacs 230–270 μ, showing rudimentary limbs.

Ripe. Ovaries densely coloured, pink. Eggs up to 110 μ, polygonal in sections, closely packed together; some have undergone, or are undergoing, maturation, germinal vesicles break down and spindles often visible in sections. Patches of active oogonia still present, but few young oocytes. Cement glands now full of clear fluid after coalescence of granules, staining pink. Ducts also now contain clear pink-staining secretion, though some granules may persist; glandular tissue in duct now reduced to a narrow strip along one side. Dark bodies near bases of ducts also contain clear fluid, which stains pink. Egg-sacs full of active cyclopid larvae, 270–290 μ, with suctorial mouth and two pairs of swimming legs. Each sac contains several hundred cyclopids. Egg-sacs of some ripe individuals have burst, releasing cyclopids, prior to spawning and extrusion of new egg-sacs.

Spawning. Old egg-sacs have been lost and larvae released. New egg-sacs formed and in process of being filled with spherical eggs. Outer wall of egg-sac probably composed of secretion from dark bodies which will be first secretion extruded. Ovaries patchy; matured eggs pass slowly down ducts in clear, sticky secretion from cement glands, and into egg-sacs, causing them to swell. Eggs in ducts compressed, cylindrical. Spawning results in the production of the spent individual, and the next generation of oocytes arise from the oogonia. Few individuals were seen at this stage.

The development of the larvae in the egg-sacs was described by Rathke (1843) and Van Beneden (1850), and is not given here in detail.

THE LIFE CYCLE AND GROWTH OF *NICOTHOË*

The cyclopid larva of *Nicothoë* (Figs. 1, 3), which is released from the egg-sac into the surrounding water, has been described by Gurney (1929). It is some 0·27–0·29 mm long, with a full complement of mouthparts, two pairs of legs and a rudimentary third pair. It has a suctorial mouth tube. The cephalothorax is as long as the rest of the body, and includes the segment of leg 1. The segments of legs 2 and 3 are free, and are followed by two legless segments and a pair of furcal rami.

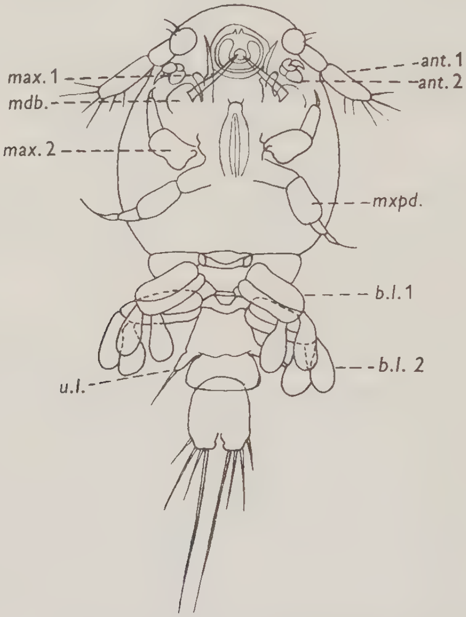


Fig. 3. Ventral view of cyclopid larva (appendages simplified). *b.l.*, biramous limb; *u.l.*, uniramous limb; other lettering as in Fig. 2.

The earliest stage of *Nicothoë* seen on lobster gills was the last-stage copepodid (Fig. 1), some 0·86–0·99 mm long, which was obtained by shaking the gills in water. It has the full complement of segments and limbs and resembles the adult in all respects except for the absence of wings and egg-sacs. Since the wings have not yet commenced development, the 6th thoracic segment abuts directly on the limb-bearing portion of the 5th. The last-stage copepodid is capable of swift, darting movements. After the last-stage copepodid is seen a whole series of stages of juveniles, showing the development of the wings, through individuals with small, bud-like growths, to those with large wings and the 6th thoracic segment well separated from the limb-bearing portion of the 5th (Fig. 1). The only increase in length after the last copepodid stage is due to the increase in size of the middle part of the wings. As growth proceeds, the gonads develop, and, usually when the wings have attained a length of some 2·0–2·2 mm (but more or less in some individuals, see Table 2), egg-sacs are extruded and the parasite becomes adult. Growth continues (the largest adult recorded had wings 4·56 mm long), and the reproductive cycle is repeated.

There is thus a gap in our knowledge of the life history of *Nicothoë*. There would seem to be a free-living stage or a phase on an intermediate host, during which one or more moults occur. There is, however, little essential difference between the mouthparts in the cyclopid and last-stage copepodid, except that, in the latter, the sucker is slightly more complicated and the 1st maxilla, whilst retaining the same form, has an extra flagellum on the anterior branch (Gurney, 1929). In both, two thoracic segments are included in the cephalothorax. The only radical changes which occur in the gap are the increase in size and the acquisition of four more segments.

Cyclopid larvae were kept for more than nine days at 11° C in filtered sea water in flasks, some with *Skeletonema costatum*, *Chlorella ovalis* or *Dunaliella primolecta* added, and some without addition. No evidence of feeding was found, and no development of the cyclopids occurred. When first released, the cyclopids swim actively and are positively phototropic, tending to congregate on the side of the container nearest the source of light. After a time they settle on the bottom of the container and become attached to it by means of their suctorial mouths. They are easily stirred to activity again. The cyclopids are capable of crawling, drawing themselves along by means of their mouths.

Other attempts to rear cyclopids were made by keeping two or three lobsters in a tank and filtering samples of surface and bottom water every 2 days. The lobsters lived for periods of from 8 to 24 days, but the larvae obtained had not developed beyond the cyclopid stage.

Plankton hauls were made over lobster beds off the west coast of Scotland and in Orkney waters between July and September. Half-metre nets, 26 or 60 meshes to the inch, were used, and hauls varied in duration from 5 to 30 min. The beds were in depths of 10–20 fathoms (18·3–36·6 m); eleven surface hauls, five midway between surface and bottom and five just above the bottom were made. Only two cyclopids were caught, both in surface hauls, and both in 26-mesh nets; neither showed any development.

In an attempt to discover a possible intermediate host, the gills and soft parts of 239 individuals representing twenty-eight species of invertebrates, and also three fish, from the lobster beds or adjacent rocky shores, were examined, mainly during the summer months. The invertebrates from the lobster beds were caught by Agassiz trawl, scallop dredge or creel. The full list of animals examined is given in an appendix. No larval *Nicothoë* were found.

Attempts were made to obtain direct infestation with cyclopid larvae of three hard and five soft (recently cast and therefore free from *Nicothoë*) lobsters in tanks of aerated sea water. The sides, but not the top, of each tank were covered with brown paper, so as to allow illumination only from above as in the sea. Active cyclopid larvae were introduced, and the lobsters were examined after times varying from 1 to 32 days. The only lobster newly infested was a soft one, left for 20 days before examination, which then carried one juvenile *Nicothoë* with wings 0·90 mm long. One isolated individual cannot be taken as indicating the successful direct settlement of a cyclopid and its subsequent moulting to a last-stage copepodid, since the lobster in question was under general circulation in the aquarium for 3 days before the commencement of the experiment, during which time it might have become infested with an intermediate larva which had been either free-living or on another host, and which had reached a stage of development at which it could moult into a last-stage copepodid. It does, however, suggest that repetition of this experiment would be valuable.

THE MALE OF *NICOTHOË*

The only individuals definitely recognized, and described and figured by previous workers, have all been females. Van Beneden (1850) described a specimen which he called a male, but Claus (1860) said that this did not even belong to the species *Nicothoë astaci*. From the description given by Van Beneden, it was almost certainly the harpacticoid copepod *Tisbe elongata*, which is frequently found on the gills of lobsters.

Claus (1860) found an individual without wings, which, from his description, was a last-stage copepodid. He concluded, however, that since the body had a full complement of segments, it was an adult, sexually mature form, and so must be a male. He supported his conclusion by quoting Rathke (1843), who described two types of larvae as being differentiated early in the egg-sac, the wings of the female appearing at a very early stage. The present investigation has shown, however, that all cyclopid larvae are of the same type (Figs. 1, 3), all with suctorial mouths, and show no sign of wings, which first appear after the last copepodid stage. The complete series of stages, from last-stage copepodids without wings, through juveniles with small wings to adults with large wings (Fig. 1), makes it certain that the copepodids develop into females.

Quidor (1906) examined five last-stage copepodids, and said that four of them had testes in various stages of development in the thorax and genital segment. The remaining one, which had no testes, he described as an immature female. In the present study 100 last-stage copepodids, taken at various times of the year, were stained with Ehrlich's acid haematoxylin and eosin and examined microscopically, but no testes were found. On the other hand, each had a pair of curved spermathecae, full of spermatozoa, in the

1st abdominal segment. These were also seen, still containing sperm, in juveniles and adults, opening into the genital duct near its opening. It would thus appear that females are impregnated at a very early stage, before settling on the lobster, and retain the spermatozoa for fertilizing each successive generation of eggs.

The males presumably develop from cyclopids during the gap in the life history between the cyclopid and last copepodid stages, but, unlike the females, they do not settle on the lobster. In addition to the last-stage copepodids, stained preparations of cyclopid larvae from six *Nicothoë* and stained sections of thirty-five juveniles and adults were examined, but no trace of a testis was found.

THE RELATIONSHIP BETWEEN *NICOTHOË* AND THE MOULTING OF THE LOBSTER

The most important moulting period of the lobster in Scottish waters is May–August, mature males casting every year and mature females every second year. Thomas (1958) has recently found some evidence of a subsidiary moulting season in younger lobsters in November. Observation of ten lobsters in the laboratory has shown that, at the moult, the outer covering of the gills is shed, and the parasites with it, leaving the gills clean. *Nicothoë* makes no attempt to leave the gills, but simply dies.

The preserved lobsters in each of the Orkney and Bernera samples were divided into three groups according to the state of the shell: soft (very recently moulted, free from epizoons), fairly soft (beginning to harden again, usually carries small epizoons, e.g. *Spirorbis*, *Pomatoceros*, *Balanus*), and hard (quite firm again, usually carries large epizoons). Their gills, whose outer layers are cast with the shell, are correspondingly soft, fairly soft and hard. The three groups are not represented in the samples in the same proportions as in nature; the samples contain a higher proportion of soft and fairly soft lobsters owing to the greater mortality among them than among hard ones during transit and storage.

Table 2 shows the numbers of last-stage copepodids, juveniles and adults of *Nicothoë* on the three types of lobsters in all the Orkney and Bernera samples, together with the mean lengths of their wings. The wings were measured from the tip to the junction of the anterior edge with the cephalothorax. The two wings of an individual are usually approximately equal in size, but, where this was not so, the longer one was measured. The length of the wing is the best index of growth owing to the small increase in body length after the last copepodid stage (p. 8).

Last-stage copepodids were found in most months of the year (none were found in September and no samples were obtained in March and May), and they occurred on soft, fairly soft and hard lobsters alike. Juvenile *Nicothoë*

TABLE 2. NUMBERS AND WING-LENGTHS OF LAST-STAGE COPEPODIDS, JUVENILES AND ADULTS ON LOBSTERS OF THE THREE SHELL-TYPES

(For convenience, the samples are considered as though taken in one year.)

			Mean nos. per lobster			Length of wings			
						Juveniles		Adults	
Month	State of shell	No. of lobsters	L.-s. copeps.	Juvs.	Adults	Mean length	Size range	Mean length	Size range
				A. Orkney, 1954–55					
April	Soft	—	—	—	—	—	—	—	—
(26. iv. 55)	Fairly soft	1	—	10·0	4·0	1·94	1·20–1·49	2·12	1·94–2·41
	Hard	10	1·7	0·5	167·6	1·70	1·38–2·11	2·82	1·51–4·04
June	Soft	1	—	—	—	—	—	—	—
(7. vi. 55)	Fairly soft	—	—	—	—	—	—	—	—
	Hard	14	3·1	2·9	49·6	1·64	0·09–2·15	2·74	1·72–4·30
August	Soft	—	—	—	—	—	—	—	—
(2. viii. 55)	Fairly soft	6	0·7	60·8	0·5	1·72	0·04–2·71	3·11	2·80–3·40
	Hard	16	—	0·2	31·1	2·05	1·94–2·15	2·80	1·81–4·13
September	Soft	7	—	29·0	0·9	2·00	1·03–2·67	2·16	1·81–2·37
(21. ix. 54)	Fairly soft	12	—	1·8	3·4	1·97	0·99–2·58	2·55	1·72–3·40
	Hard	6	—	—	23·7	—	—	2·66	1·63–3·70
November	Soft	—	—	—	—	—	—	—	—
(8. xi. 55)	Fairly soft	14	—	1·0	9·9	2·12	1·72–2·37	2·96	1·81–4·09
	Hard	5	—	1·3	80·8	1·58	1·51–1·98	3·29	2·11–4·47
December	Soft	—	—	—	—	—	—	—	—
(14. xii. 54)	Fairly soft	3	—	0·3	25·7	1·42	1·42	2·79	2·37–3·48
	Hard	9	—	—	20·0	—	—	2·66	1·76–3·66
				B. Bernera, 1955–56					
January	Soft	—	—	—	—	—	—	—	—
(26. i. 56)	Fairly soft	5	0·2	1·8	9·2	1·85	0·95–2·32	2·59	1·85–3·48
	Hard	3	—	—	103·7	—	—	2·81	1·85–3·87
February	Soft	—	—	—	—	—	—	—	—
(8. ii. 56)	Fairly soft	3	0·3	23·7	24·3	1·97	1·28–2·58	2·53	1·59–3·83
	Hard*	8	0·5	2·0	81·2	1·44	0·43–2·36	2·74	1·55–4·13
April	Soft	3	—	10·7	4·0	1·88	0·90–2·32	2·31	1·89–2·32
(12. iv. 56)	Fairly soft	3	—	26·0	171·3	2·06	1·46–2·75	2·78	1·72–3·87
	Hard	2	—	1·0	4·0	1·26	0·77–1·76	2·71	2·15–3·48
June	Soft	1	6·0	4·0	—	1·06	0·73–1·33	—	—
(19. vi. 56)	Fairly soft	4	0·2	41·0	15·5	2·12	0·82–2·92	2·45	1·98–3·05
	Hard	5	1·6	0·4	112·2	1·66	1·63–1·68	2·99	2·28–4·56
July	Soft	1	9·0	180·0	—	1·05	0·09–1·89	—	—
(24. vii. 56)	Fairly soft	6	4·8	47·2	46·0	1·63	0·04–2·80	2·52	1·72–3·66
	Hard	2	—	1·5	266·5	1·99	1·59–2·58	2·94	2·02–4·30
August	Soft	—	—	—	—	—	—	—	—
(24. viii. 56)	Fairly soft	6	0·2	3·2	122·7	2·03	1·16–2·49	2·57	1·29–4·00
	Hard*	5	0·8	0·6	83·2	1·91	1·51–2·15	2·65	1·63–3·70
October	Soft	—	—	—	—	—	—	—	—
(4. x. 55)	Fairly soft	8	2·2	22·5	38·4	1·15	0·09–2·32	2·49	1·38–3·66
	Hard*	3	—	0·3	90·7	0·22	0·22	2·88	1·94–4·21
November	Soft	—	—	—	—	—	—	—	—
(2. xi. 56)	Fairly soft	10	0·4	9·9	55·6	2·06	1·16–2·75	2·77	1·36–4·47
	Hard	3	—	5·0	174·7	1·76	1·20–2·75	2·68	1·59–4·26
December	Soft	—	—	—	—	—	—	—	—
(7. xii. 55)	Fairly soft	15	17·9	9·3	19·0	1·67	0·09–1·76	2·85	1·89–4·26
	Hard*	4	3·8	1·5	122·2	0·77	1·29–2·24	3·07	2·32–4·21

Each sample marked * excludes one hard lobster with badly damaged gills, which carried more than the usual numbers of juvenile *Nicothoë*, as below (see text):

February	Hard	1	—	10	390	1·87	1·68–2·06	2·69	1·72–3·74
August	Hard	1	2	14	508	0·88	0·22–1·89	2·70	1·85–3·78
October	Hard	1	1	40	288	1·69	0·69–2·11	2·93	1·72–3·87
December	Hard	1	16	44	756	1·95	1·29–2·24	3·07	2·32–4·31

were found in all samples, but, with only four exceptions, were present in numbers only on lobsters which had recently moulted (soft and fairly soft). Hard lobsters had the most, and also the largest (adult) *Nicothoë*. It appears probable that the last-stage copepodid cannot pierce a gill filament if the latter is hard. If, on the other hand, the gills are soft, the parasite attaches itself by its suctorial mouth to a filament, which it pierces by means of its mandibles. Feeding on the host's blood then results in the growth of the wings and development, through the juvenile, to the adult state.

TABLE 3. PERCENTAGE OF INDIVIDUALS OF *NICOTHOË* IN EACH STAGE OF DEVELOPMENT—HARD LOBSTERS

(For convenience, the samples are considered as though all taken in one year.)

	Gonad stage						
Month	Empty	De-veloping	Half-full	Full	Ripe	Spawn-ing	No. examined
		A. Orkney, 1954–55					
April (26. iv. 55)	46·1	13·9	3·1	11·0	23·5	2·4	1185
June (7. vi. 55)	18·5	25·6	15·8	15·7	23·1	1·3	671
August (2. viii. 55)	15·1	33·0	23·0	13·0	15·9	—	339
September (21. ix. 54)	26·1	22·5	21·8	25·4	4·2	—	142
November (8. xi. 55)	12·4	27·2	14·8	15·1	28·0	2·5	404
December (14. xii. 54)	15·0	8·4	16·1	52·2	7·2	1·1	180
		B. Bernera, 1955–56					
January (26. i. 56)	3·9	31·5	28·0	21·2	13·2	2·2	311
February (8. ii. 56)	11·7	25·5	21·0	22·5	19·3	0·1	1040
April (12. iv. 56)	4·8	16·1	28·2	27·4	23·0	0·6	522*
June (19. vi. 56)	20·9	24·4	11·2	13·5	30·0	—	561
July (24. vii. 56)	32·3	15·2	13·7	17·1	21·7	—	533
August (24. viii. 56)	27·7	20·4	5·4	21·6	22·2	2·7	924
October (4. x. 55)	47·5	18·0	5·9	16·3	12·1	0·2	560
November (2. xi. 56)	23·7	18·1	12·0	14·1	31·1	1·0	524
December (7. xii. 55)	27·0	20·6	15·4	15·4	21 4	0·2	1245

* Includes two lobsters not quite hard.

The four exceptions referred to above, lobsters with hard shells which carried respectively 10, 14, 40 and 44 juvenile *Nicothoë*, all had badly damaged gills. It is probable that the last-stage copepodids became attached, and the damage to the gills permitted them to suck the host's blood without the necessity of penetrating the filaments.

One lobster, caught in December 1955, was noteworthy in that, while the shell was hard, and the gills on the left-hand side were hard and greeny brown, those on the right-hand side were soft, clean and white. The left-hand gills carried 100 adult *Nicothoë* and no juveniles, while the right-hand gills carried no adults and 23 juveniles.

There is a seasonal rhythm in the release of cyclopid larvae of *Nicothoë*. Table 3 shows the percentages of adult *Nicothoë* in each of the six stages of gonad development (p. 7). Since lobsters of the three shell groups are not present in the same proportion as in nature, only hard ones, which are in the majority in nature even in the moulting season, were considered. Adults in the ripe and spawning stages indicate the release, or impending release, of cyclopid larvae. Since these stages were present in all samples taken, release of larvae occurs throughout the year. Two peaks were shown in the release of cyclopid larvae in both Orkney and Bernera lobsters (Fig. 4). The first peak, in May or June, occurred at the beginning of the main moulting period, and the percentage remained relatively high throughout this period, while the second peak, in November, coincided with the subsidiary moulting period.

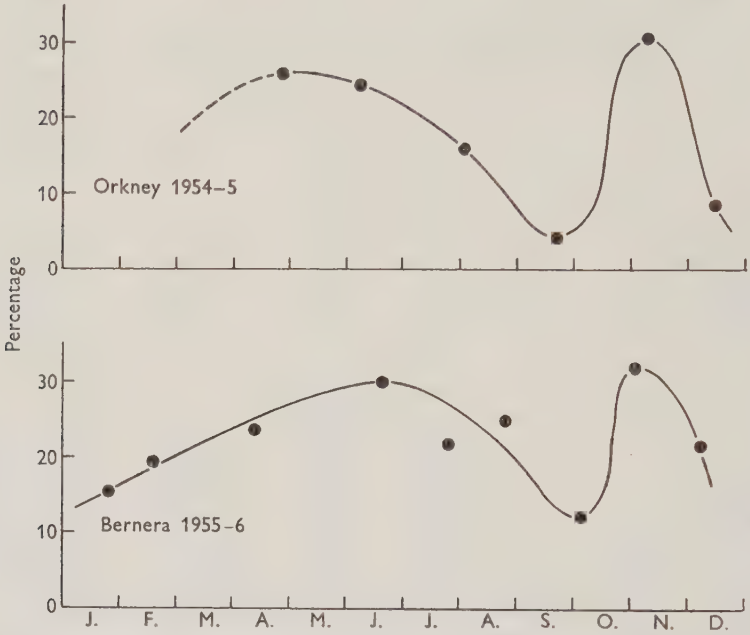


Fig. 4. Percentages of adult *Nicothoë* in ripe and spawning stages: Orkney, 1954–5; Bernera, 1955–6. See Table 3.

It appears, then, that *Nicothoë* can infest only recently cast lobsters, but it can do so at any time of the year. Most infestation will, however, presumably occur during the main moulting periods, when the highest proportion of soft lobsters is present, and when most cyclopid larvae are released.

I wish to thank Dr H. J. Thomas for helpful suggestions and for reading the manuscript. I am grateful to Dr J. H. Fraser for help in interpreting the segmentation and in identifying larvae, and to Dr S. Symmonds for advice on microscopic technique. The kindness of Messrs D. Meil and Sons, Kirkwall, and of Crofters' Supply Agency, Oban, in providing material, is gratefully acknowledged.

SUMMARY

The only individuals of *Nicothoë astaci* so far definitely recognized have been female.

Concurrent series of changes take place in the ovaries and egg-sacs, so that when the gonads are full of ripe eggs, and are ready to spawn, the egg-sacs either contain active cyclopid larvae or have just released them. After the release of the cyclopid into the sea there is a gap in our knowledge of the life cycle, the next stage known being the much larger last-stage copepodid on the gills of the lobster. From this a series of changes can be traced, with the appearance of wings and the development of gonads, through juveniles (which have never spawned) to adults with egg-sacs. All attempts to bridge the gap have failed; cyclopid larvae kept alive for up to 9 days showed no sign of development, and no intermediate stages have been found, either free-living in the plankton or parasitic on the lobster or other animals. Attempts to parasitize lobsters by means of cyclopid larvae have failed with one exception whose significance is doubtful.

Individuals described as males by previous workers either belonged to another genus or were almost certainly last-stage copepodids. All last-stage copepodids examined had spermathecae containing sperm, and must have been impregnated at a very early stage, the male presumably developing in the gap between cyclopid and last-stage copepodid, though all the cyclopids were of one type. The sperm are retained and fertilize successive generations of eggs.

When the lobster moults the *Nicothoë* are shed with the old shell and die, and re-infestation by settlement of last-stage copepodids can only occur in significant numbers shortly after the moult, before the gills become hard again. There is a seasonal rhythm in the release of cyclopid larvae by the adult females, there being two peaks, of which one coincides with the lobster's main moulting season (May–August) and the other with a subsidiary moulting period of younger lobsters (November).

REFERENCES

Claus, C., 1860. Zur Morphologie der Copepoden. 2. Über den Bau von *Nicothoë*. *Würzburg naturwiss. Z.* Bd 1, pp. 22–5.

Gurney, R., 1929. The larva of *Nicothoë astaci* and its systematic position. *J. mar. biol. Ass. U.K.*, Vol. 16, pp. 453–9.

Leigh-Sharpe, W. H., 1926. *Nicothoë astaci* (Copepoda) with a revision of the appendages. *Parasitology*, Vol. 18, pp. 148–53.

Quidor, A., 1906. Sur le mâle et l'appareil suceur de *Nicothoë astaci*. *C.R. Acad. Sci., Paris*, T. 142, pp. 465–8.

Rathke, H., 1843. Beiträge zur Fauna Norwegens. Crustacea. *Nova Acta Leop. Carol.*, Bd 20, pp. 1–264.

Thomas, H. J., 1958. Some seasonal variations in the catch and stock composition of the lobster. *J. Cons. int. Explor. Mer*, Vol. 24, pp. 147–54.

Van Beneden, P. J., 1850. Mémoire sur le développement et l'organisation des *Nicothoë*. *Mém. Acad. R. Belg.*, T. 24, pp. 3–28.

APPENDIX

Animals examined for intermediate stages of Nicothoë

		No. examined
Porifera	*Halichondria panicea*	4
Coelenterata	*Aurelia aurita*	1
	Actinia equina	9
Annelida	*Nereis pelagica*	6
Crustacea	*Balanus* sp. (from lobsters' shells)	30
	Gammarus sp.	7
	Pandalus montagui	8
	Palaemon serratus	2
	Crangon vulgaris	1
	Nephrops norvegicus (trawled nearby)	10
	Munida bamffica	1
	Eupagurus bernhardus	4
	Portunus puber	39
	Carcinus maenas	17
	Cancer pagurus	22
	Hyas araneus	1
	Inachus dorsettensis	2
Mollusca	*Patella vulgata*	7
	Gibbula cineraria	2
	Littorina littorea	10
	Nucella lapillus	12
	Mytilus edulis	8
	Chlamys opercularis	10
Echinodermata	*Astropecten irregularis*	1
	Porania pulvillus	4
	Asterias rubens	11
	Ophiura texturata	2
	Psammechinus miliaris	8
Pisces	*Zoarces viviparus*	1
	Cottus bubalis	2

J. mar. biol. Ass. U.K. (1959) 38, 17–25
Printed in Great Britain

THE LARVAL AND POST-LARVAL STAGES OF *GYMNAMMODYTES SEMISQUAMATUS* (JOURDAIN)

By JANE CAMERON

The Marine Biological Station, Port Erin

In the course of investigations (1955–8) into the Ammodytidae of the south of the Isle of Man, five species of sand eel were identified: *Gymnammodytes semisquamatus* (Jourdain, 1879), the Smooth Sand Eel; *Ammodytes lanceolatus* Lesauvage, 1825, the Greater Sand Eel; *A. immaculatus* Corbin, 1950; *A. tobianus* Linnaeus, 1758 (=*A. lancea* Cuvier), the Lesser Sand Eel; *A. marinus* Raitt, 1934.

Five different types of post-larvae were obtained and investigated. Four of these were the post-larvae of the above four *Ammodytes* species and the fifth type proved to be *Gymnammodytes semisquamatus*.

A. lanceolatus and *A. marinus* post-larvae were identified according to Einarsson (1951) and Corbin & Vati (1949), and *A. tobianus* was identified according to Einarsson (1955). The results agreed with Einarsson's conclusions on the identification of these post-larvae. Very few post-larvae of *A. immaculatus* (Corbin & Vati, 1949—*Ammodytes* Species IV) were obtained and these were identified by vertebral counts as they are externally very similar to *A. lanceolatus* post-larvae.

Previous confusion in the identification of *Ammodytes* post-larvae has now been cleared up by the investigations of Einarsson (1951, 1955). In 1949 Corbin & Vati published a description of four Ammodytidae post-larval types. These were ascribed to the species *A. lanceolatus*, *A. marinus*, *A. tobianus* and *Ammodytes* Species IV (=*A. immaculatus* Corbin, 1950*a*). Corbin noted a marked discrepancy between vertebral counts made on the post-larvae ascribed to *A. tobianus* and counts made on a sample of adult *A. tobianus*. Subsequent investigations into *Ammodytes* post-larvae by Einarsson (1951) proved that the *A. marinus* post-larvae of Corbin & Vati (and of Kändler, 1941) were correctly identified, but that the post-larval type which Corbin & Vati called *A. tobianus* was in fact *A. lanceolatus*.

The post-larval type ascribed by Corbin & Vati (also by Ford, 1920; Kändler, 1941) to *A. lanceolatus* therefore belonged to some other species.

Einarsson (1951) had no opportunity to examine intact specimens of this post-larval type, but he discussed some of its peculiar features, distinct from other Ammodytidae post-larvae, and suggested the possibility of its being the young of *G. semisquamatus*: 'The data on post-larvae of *A. lanceolatus* must be referred to another species of Ammodytidae, not definitely known as yet, but most probably *G. semisquamatus.*' After the present work was completed it was brought to my notice that the Council of the Marine Biological Association of the United Kingdom reported (*J. mar. biol. Ass. U.K.*, 1954, Vol. 33, p. 771) that Mr P. G. Corbin obtained several artificial fertilizations of the eggs of *G. semisquamatus* in 1953, and that the larvae from the artificial fertilizations provided independent confirmation of Einarsson's identification.

Post-larvae of this type are abundant in the plankton off the south of the Isle of Man from May to September, and it has been possible to obtain large numbers for investigation.

A series of larvae and post-larvae from 3 mm through the late post-larval to juvenile stages of 40–50 mm have been examined and there is no doubt that these post-larvae are *G. semisquamatus.*

Post-larval stages of *G. semisquamatus* can be identified by external characters alone. Several identifying characters were found to be important. These come under the following headings and are discussed below: (1) the pattern of pigmentation, (2) 'teeth' on the upper jaw, (3) protrusibility of the upper jaw, (4) vomerine teeth, (5) juvenile and adult characteristics.

During the course of post-larval development the above characters overlap successively so as to give a complete sequence of specific characters from the smallest larva obtained (3 mm) to juvenile fish of 30–50 mm in which adult characteristics are already developing.

CHARACTERS OF POST-LARVAL *GYMNAMMODYTES SEMISQUAMATUS*

The pattern of pigmentation

The nomenclature of the post-larval pigmentation is shown in Text-fig. 1 (after Corbin & Vati, 1949).

The presence of a ventral fin-membrane pigment row is specific to the early post-larvae of *G. semisquamatus*. It occurs in the smallest specimens obtained and persists through all stages until about 19 mm. In the post-larvae of the *Ammodytes* species there is no ventral fin-membrane pigment at any stage.

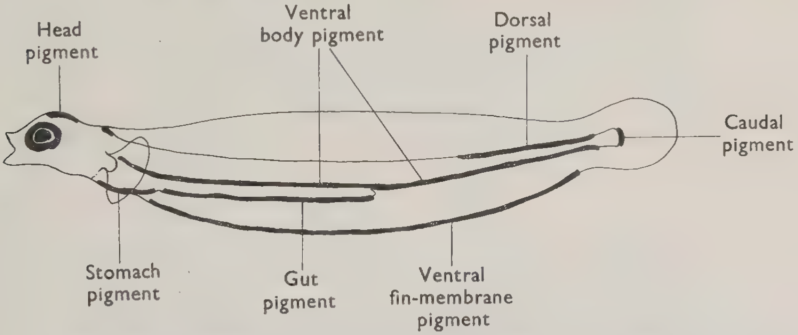
'Teeth' on the upper jaw

At about 7 mm the post-larva develops a row of tooth-like structures along the outer edge of the rim of the upper jaw (pre-maxilla). These are pointed, shaped like thorns and can be seen without staining with alizarin. They are found only in the post-larvae of *G. semisquamatus* and not in the post-larvae of the *Ammodytes* species. They persist until the post-larva is 20–25 mm long, by which length the growing tissues around the lips have obliterated the larval 'teeth'.

Protrusibility of the upper jaw

This becomes detectable at 8–10 mm and from then on it is a very important feature. Protrusibility becomes increasingly more marked in increasingly larger post-larvae and this therefore rules out the possibility of the post-larvae belonging to the species *A. lanceolatus* or *A. immaculatus*, both of which species have a non-protrusible jaw.

In juvenile and adult *G. semisquamatus* the upper jaw is very protrusible. When the upper jaw is shot forward during feeding the whole mouth forms a long tube. The mouth can be protruded farther than that of either *A. tobianus* or *A. marinus* and the mechanism of protrusion and the shape of the head and jaws are quite characteristic in juvenile and adult *G. semisquamatus*. These adult features of the head and jaws can be recognized to be developing in the post-larva of 18 mm or earlier. From there they can be traced through different stages to the juvenile.



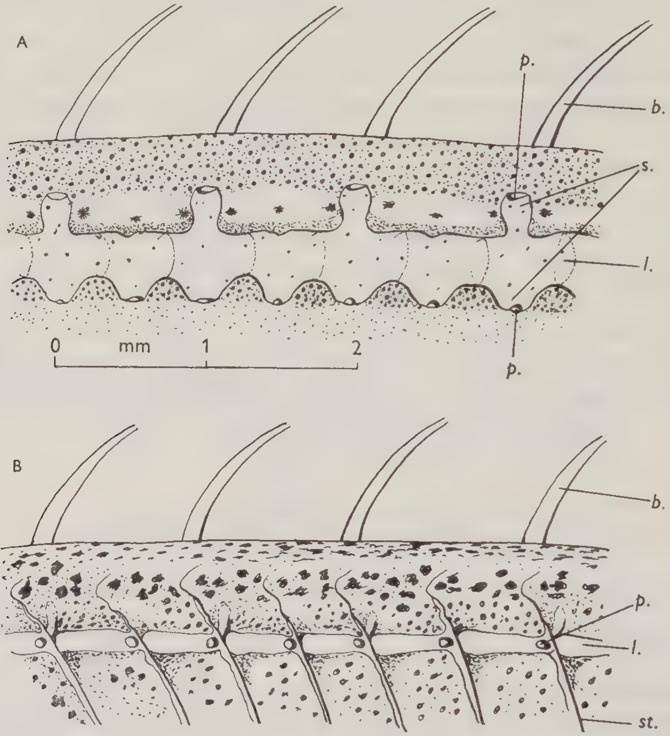
Text-fig. 1. Diagram showing the nomenclature of the pigment rows. (After Corbin & Vati, 1949.)

Vomerine teeth

A pair of sharp downward- and outward-curving teeth begin to develop on the post-larval vomer (figured by Einarsson, 1951) at about 12–15 mm length. These were previously taken to be diagnostic characters of *A. lanceolatus*, but their structure and the shape of the vomer is different from that in the *A. lanceolatus* post-larva (see Einarsson, 1951, pp. 24–5, figs. 6, 7). Moreover, the vomerine teeth of *A. lanceolatus* are not visible until the post-larva has reached 20–25 mm in length; they persist throughout development and are present in the adult. The vomerine teeth of post-larval *G. semisquamatus* do not persist in the adult but they remain until after the ventral fin-membrane pigment row and the 'teeth' on the upper jaw have gone. They disappear when the fish is between 40 mm and 50 mm long, but by then it is a late post-larva-to-juvenile, some of the adult characteristics being already present and the fish recognizable as *G. semisquamatus* by the criteria applied to adults.

The important identifying characters of juvenile and adult *G. semisquamatus* are as follows.

(1) The structure of the lateral line is characteristic (see Text-fig. 2). Transverse side-channels of the lateral-line canal lead to the pores, in contrast to the lateral line of the *Ammodytes* species which is linear (Duncker & Mohr, 1939, p. 11, fig. 1; Jourdain, 1879, figs. 13, 14). The scales along the lateral line are not pierced by pores as they are in the *Ammodytes* species.



Text-fig. 2. The structure of the lateral line (lateral view). A, *Gymnammodytes semisquamatus*. B, *Ammodytes marinus*. *b*, base of dorsal fin-ray; *l*, lateral line canal; *p*, pore; *s*, side-channels; *st*, skin-folds or striae.

(2) The upper jaw is markedly protrusible.

(3) There are no vomerine teeth.

(4) The body is smooth and unstriated.

(5) The ventro-lateral skin-fold extends from the base of the pectoral fin to a point level with, or just beyond, the tip of the pectoral fin when this is pointing towards the tail.

(6) The colour of the head and back is golden-brown or dark purplish brown, while the sides and belly are silvery and iridescent.

A diagnosis of the species *G. semisquamatus* from the newly hatched larva, up to the stage at which it can be recognized by criteria applied to the adult, is given below.

DIAGNOSIS

Larvae, 3–4 *mm* (Pl. I, figs. 1, 2)

Ventral fin-membrane pigment row present. No 'teeth' on upper jaw. Upper jaw cannot be protruded. No vomerine teeth.

Yolk-sac present, partially absorbed. The yolk-sac may be completely absorbed at 4 mm. No dorsal or head pigment present. Stomach pigment consists of four, five or six brown, large and many-branched melanophores along the ventral mid-line of the yolk-sac, with two or more melanophores on the sides of the yolk sac. Ventral body pigment shows clearly pre- and post-anally. The ventral fin-membrane pigment row extends from the stomach region nearly to the tail.

Post-larvae, 5–7 *mm* (Pl. I, figs. 3, 4)

Ventral fin-membrane pigment row present. 'Teeth' become visible at 7 mm (Pl. I, fig. 4). Upper jaw cannot be protruded. No vomerine teeth.

Yolk-sac absent. There may be a single median melanophore over the occipital region of the head. One or two dorsal melanophores are present directly in front of the tail. There are two caudal melanophores ventral to the tip of the notochord. The first rudiments of caudal fin-rays are visible at 6–7 mm.

The notochord is straight.

Post-larvae, 8–10 *mm*

Ventral fin-membrane pigment row present. 'Teeth' present on upper jaw. Upper jaw can be protruded at 9–10 mm. Vomerine teeth not visible.

The notochord begins to turn up posteriorly at 9 mm, and at this length caudal fin-rays are beginning to develop. There may now be three to five dorsal melanophores at the posterior end, but usually only two or three. Pigment is otherwise as before.

Post-larvae, 11–19 *mm* (Pl. I, fig. 5; Pl. II, figs. 1–3)

Ventral fin-membrane pigment row present. 'Teeth' present on upper jaw. Upper jaw protrusible and increasingly so with increasing size of fish. Vomerine teeth visible from 12 to 15 mm.

The notochord is turned up and the tail is in the adult position at 11 mm (Pl. I, fig. 5). Also at 11 mm five or six dorsal fin-rays can be seen to be developing midway between anus and tail. Below these, five or six anal fin rays are developing. By 19 mm all anal fin-rays are present, and dorsal fin-rays extend from the tail farther forward than the level of the anus.

Head pigment begins to show at 11–12 mm—two stellate melanophores at first, later up to five. Dorsal pigment is now at maximum for post-larvae. It consists of never more than five melanophores all close to the tail. The dorsal pigment in the post-larva rarely extends as far as half-way between the tail and the level of the anus. The ventral body pigment is becoming obscured pre-anally by the downward growth of the abdomen walls but can still be seen in side view as about seventeen dark spots. Stomach pigment is now made up of four to five small compact melanophores. Gut pigment consists of about seventeen very small black dots extending from stomach to anus. Both stomach and gut pigment are usually lost at a post-larval length of

16–17 mm. Ventral fin-membrane pigment becomes more reduced as anal fin-rays develop. By 19 mm there are only a few ventral fin-membrane melanophores remaining—all anterior to the anus. In fresh or freshly preserved specimens of 16–19 mm there are very small well-defined orange chromatophores along the ventral surface of the gut and stomach, and lines of these chromatophores extend forwards between the gills (not shown in illustration).

Post-larvae, 20–24 mm (Pl. II, fig. 4)

Ventral fin-membrane pigment row absent. 'Teeth' present on upper jaw. Upper jaw protrusible. Vomerine teeth present.

Dorsal fin-rays extend to half-way between head and anus. There is more pigment on the head, consisting of four or more stellate melanophores. The occipital melanophore remains and there is usually one melanophore or a small cluster on the operculum behind the eye. There is now no trace of stomach or gut pigment. The dorsal pigment is the same as before. Ventral body pigment is still visible but obscure in the pre-anal region. There is a single, central caudal melanophore. Ventral orange chromatophores are as above.

Post-larvae, 25–29 mm

'Teeth' no longer visible on upper jaw. Upper jaw protrusible. Vomerine teeth present.

The full number of dorsal fin-rays are present. The dorsal fin extends forward to a point level with the tip of the pectoral fin when this is turned to the posterior.

Ventral body pigment is now very obscured behind the abdomen wall but is still well-marked post-anally. There is now more head pigment.

Post-larvae, 30–37 mm

No specimens examined.

Post-larva-to-juvenile, 38–47 mm (Pl. II, fig. 5)

Upper jaw markedly protrusible. Vomerine teeth present. Vomerine teeth are reduced but still visible at 47 mm.

Brown juvenile pigment is developing especially on the top of the head, around the jaws, and along the dorsal mid-line at the base of the dorsal fin on each side. Brown chromatophores are also present along the lateral lines, at the base of the caudal fin and along the caudal fin-rays.

The lateral lines are close to the base of the dorsal fin, and extend on either side of the dorsal fin from a point level with the posterior edge of the operculum and the base of the pectoral fin to a small distance in front of the tail. The lateral lines already show adult characteristics.

Juveniles, 48–50 *mm* (Pl. II, fig. 6)

Upper jaw markedly protrusible. Vomerine teeth lost.

The fish are otherwise as above (38–47 mm) with increasing juvenile-adult pigment forming along the lateral lines (large brown chromatophores), on the head, the sides of the body and on the caudal fin.

Juveniles, 50–60 *mm*

The juveniles resemble adults in colour and proportions.

NUMBER OF VERTEBRAE

Vertebral counts were made on 150 adult *G. semisquamatus* and also on 33 post-larvae. The post-larvae were stained with alizarin and afterwards cleared. The urostyle was included in all counts.

The results are given in Tables 1 and 2, together with the results of Corbin & Vati (1949) and Corbin (1950*b*) which are the only vertebral counts of *G. semisquamatus* I have been able to obtain. The Celtic Sea post-larvae of Corbin & Vati (1949) were ascribed to *A. lanceolatus* but are now known to have been *G. semisquamatus* (see p. 17 above).

TABLE 1. RESULTS OF VERTEBRAL COUNTS ON *GYMNAMMODYTES SEMISQUAMATUS*

Adults, all age-groups		Post-larvae	
No. of vertebrae	No. of specimens	No. of vertebrae	No. of specimens
65	1	65	1
66	15	66	4
67	53	67	13
68	67	68	11
69	13	69	4
70	1	70	·
Total	150		33
Mean no. of vertebrae	67·53 ±0·069		67·39 ±0·169

TABLE 2. THE NUMBER OF VERTEBRAE IN *GYMNAMMODYTES SEMISQUAMATUS*

Area and age of specimens	Author	No. of speci- mens	Range of no. of vertebrae	Mean no. of vertebrae	Standard error of mean
West Scotland and northern North Sea adults	Corbin & Vati (1949)	51	65–70	68·16	±0·144
Celtic Sea post-larvae	Corbin & Vati (1949)	52	64–70	68·08	±0·082
Plymouth adults	Corbin (1950*b*)	108	66–72	68·44	±0·098
Irish Sea adults	Present records	150	65–70	67·53	±0·069
Irish Sea post-larvae	Present records	33	65–69	67·39	±0·169

The range of number of vertebrae of the Irish Sea specimens is within the ranges for previous records, but the mean number of vertebrae for the Irish Sea is slightly lower than previous records.

SUMMARY

Larval and post-larval stages of *Gymnammodytes semisquamatus* are described from recently hatched larvae up to juvenile stages.

Late post-larvae and juveniles are identified as *G. semisquamatus* by the criteria applied to adults.

The Ammodytidae post-larvae ascribed by Ford (1920), Kändler (1941) and Corbin & Vati (1949) to *Ammodytes lanceolatus*, are shown to be identical with post-larval stages of *G. semisquamatus.*

G. semisquamatus post-larvae can be isolated from all other Ammodytidae by the following external characters which, occurring in combination, are diagnostic: (1) presence of ventral fin-membrane pigment row, (2) tooth-like structures on the edge of the upper jaw, (3) protrusible upper jaw, (4) larval vomerine teeth lost in development when the fish is between 40 and 50 mm long, and (5) adult characters of *G. semisquamatus* which are present in the late post-larval and juvenile stages.

During the course of post-larval development the above characters overlap successively giving a complete series of specific characters from the smallest larva obtained (3 mm) to juvenile fish of 30–50 mm in which adult characteristics are developing.

Vertebral counts of post-larvae of *G. semisquamatus* taken near the Isle of Man are consistent with those of adults of the same area.

The mean number of vertebrae of adult *G. semisquamatus* from this area was 67·53 ± 0·07. This is lower than previous records for other areas which range from 68·08 ± 0·08 to 68·44 ± 0·1.

REFERENCES

Corbin, P. G. 1950*a*. *Ammodytes immaculatus*, a new species of sand-eel found in European seas. *Nature, Lond.*, Vol. 166, pp. 525–26.

—— 1950*b*. The occurrence of the smooth sand-eel *Gymnammodytes semisquamatus* (Jourdain) in the Plymouth area, with notes on *G. cicerelus* (Rafinesque), and *G. capensis* (Barnard). *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 83–95.

Corbin, P. G. & Vati, V., 1949. The post larval sand-eels (Ammodytidae) of the Celtic Sea and Plymouth area. *J. mar. biol. Ass. U.K.*, Vol. 28, pp. 287–313.

Duncker, G. & Mohr, E., 1939. Revision der Ammodytidae. *Mitt. zool. Mus. Berl.*, Bd. 24, Heft 1, pp. 8–31.

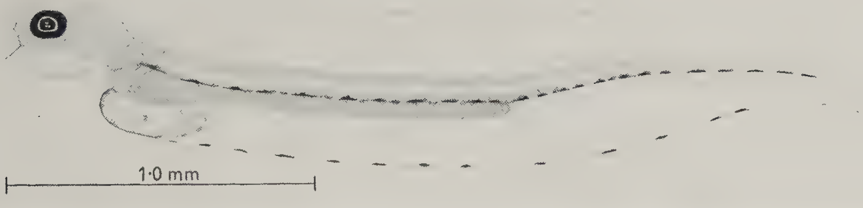
Einarsson, H., 1951. The post-larval stages of sand-eels (Ammodytidae) in Faroe, Iceland and W-Greenland waters. *Acta nat., islandica*, Vol. 1, No. 7, 75 pp.

—— 1955. On the post-larval stages of *Ammodytes lancea* Cuvier. *Acta nat. islandica*, Vol. 2, No. 1, 7 pp.

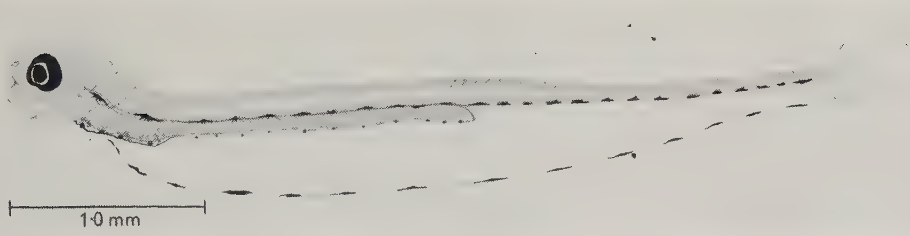
Ford, E., 1920. The post-larval stages of *Ammodytes* species captured during the cruises of S.S. *Oithona* in Plymouth waters in the year 1919. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 241–48.

Jourdain, S., 1879. Sur les *Ammodytes* des côtes de la Manche. *Rev. Sci. nat.*, Sér. 2, T. 1, pp. 203–10.

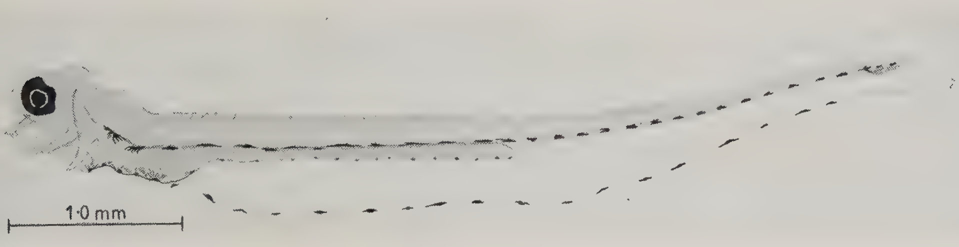
Kändler, R., 1941. Untersuchungen über Fortpflanzung, Wachstum und Variabilität der Arten des Sandaals in Ost- und Nordsee, mit besonderer Berücksichtigung der Saissonrassen von *Ammodytes tobianus* L. *Kieler Meeresforsch.*, Bd. 5, Heft 1, pp. 45–145.



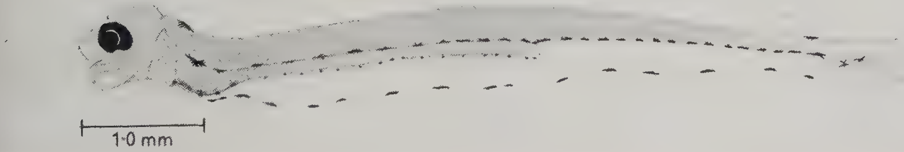
1



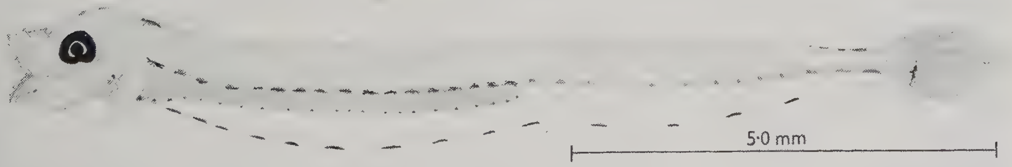
2



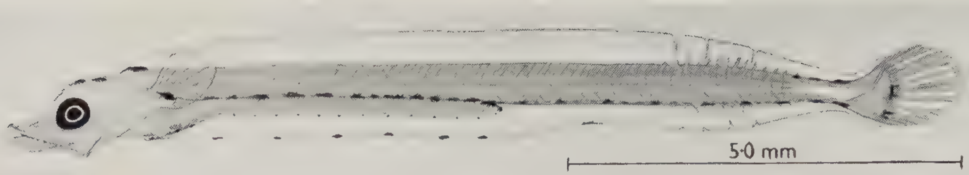
3



4

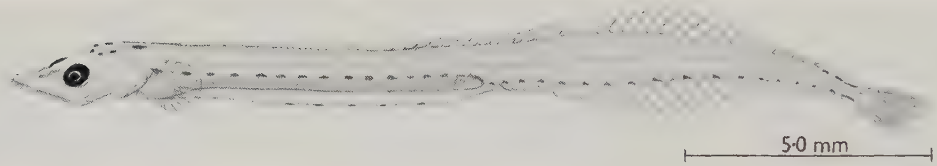


5

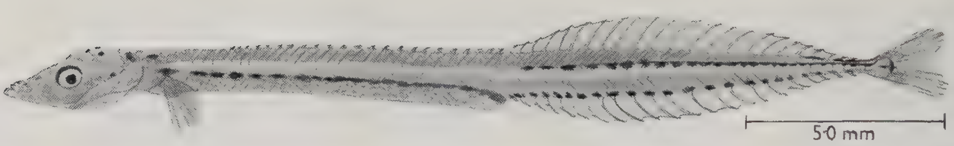


1

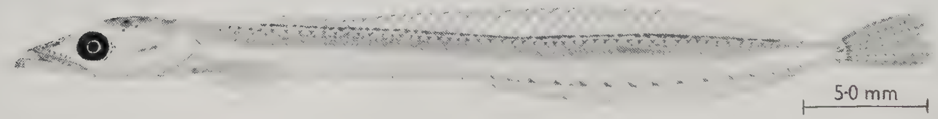
2



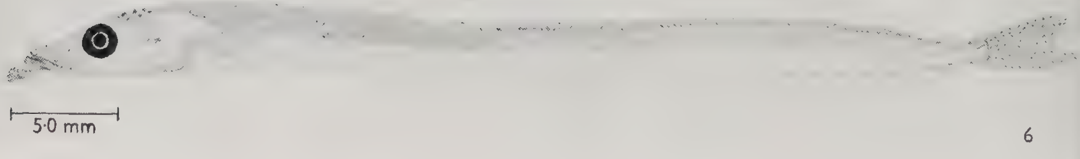
3



4



5



6

EXPLANATION OF PLATES I AND II

Development stages of *Gymnammodytes semisquamatus* larvae and post-larvae. The drawings were made from specimens preserved in formalin. Measurements represent total length.

Plate I

Fig. 1. Larva, 3 mm.
Fig. 2. Post-larva, 4·75 mm.
Fig. 3. Post-larva, 5·75 mm.
Fig. 4. Post-larva, 7·0 mm.
Fig. 5. Post-larva, 11·75 mm.

Plate II

Fig. 1. Post-larva, 12·5 mm.
Fig. 2. Post-larva, 14·0 mm.
Fig. 3. Post-larva, 19·5 mm.
Fig. 4. Post-larva, 24·0 mm.
Fig. 5. Post-larva, 38·0 mm.
Fig. 6. Juvenile, 48·0 mm.

J. mar. biol. Ass. U.K. (1959) 38, 27–32
Printed in Great Britain

A PORPHYRIN PIGMENT IN THE INTEGUMENT OF *ARION ATER* (L.)

By G. Y. Kennedy

Cancer Research Unit, University of Sheffield

MacMunn (1886) extracted a porphyrin from the integument of the starfish *Asterias rubens*—then known as *Uraster rubens*—and he considered that this and similar pigments which he had obtained from the slug *Arion empiricorum* and the coelenterates *Flabellum variabile* and *Fungia symmetrica* were identical with the haematoporphyrin of Hoppe-Seyler (1871), which was then the only porphyrin known. Dhéré & Baumeler (1928*a*) confirmed the presence of a porphyrin in *Arion empiricorum*, but did not specify which porphyrin they had found. Kennedy & Vevers (1953), working at the Plymouth Laboratory of the Marine Biological Association, found that the pigment of the integument of *Asterias rubens* L. was protoporphyrin, and in a further survey of the porphyrins of marine invertebrates (Kennedy & Vevers, 1954), they examined the integuments of two molluscs, *Aplysia punctata* Cuvier and *Duvaucelia plebeia* (Johnston), and showed that each contained uroporphyrin I. This pigment was also found by Kennedy & Vevers (1956) to be present in the integument of the tectibranch mollusc *Akera bullata* (O. F. Müller). The present paper describes the isolation and identification of the porphyrin from the integument of the black garden slug *Arion ater* (L.). *Arion empiricorum* was a synonym introduced by Férussac, Férussac & Deshayes (1819–51) to cover the numerous colour and pattern varieties of the slug more generally known as *Arion ater*. The work appears here since it is a continuation of investigations of mollusc pigments initiated in the Plymouth Laboratory, the results of which have already been published in this *Journal*.

The writer is very grateful to Mr O. Thornburn of the Cancer Research Unit, University of Sheffield, for collecting the specimens of *Arion ater* for this work.

METHOD

Twenty-seven large specimens of the black *Arion* were collected from Lodge Moor, near Sheffield, and killed by chloroform vapour. The very viscous yellow slime was removed with a cloth and the integuments dissected off. The internal surfaces of the integuments were scraped clean and the tissue chopped with scissors into 500 ml. of a mixture of absolute methanol 19 parts and concentrated sulphuric acid 1 part. The extraction was allowed to proceed overnight at room temperature, and the residue then filtered off and re-extracted for 4 h with a further 500 ml. of the methanol-sulphuric acid mixture. The extracts were pooled, diluted with an equal volume of water, cooled to 5° C, and extracted with chloroform until the hypophases were no longer fluorescent. The chloroform extract was washed with 2% sodium chloride twice, followed by six washes with distilled water, dried roughly by filtration through chloroform-soaked paper, and concentrated *in vacuo* at 60° C.

The extract was dark brown and red-fluorescent, and obviously contained a great deal of porphyrin. A sample diluted with chloroform gave the following spectrum (Hartridge):

626·7 572·0 537·3 500·7 mμ.

This spectrum very closely resembles that given by uroporphyrin.

CHROMATOGRAPHY

Column chromatography

The chloroform extract of the pigment was evaporated to dryness and the residue redissolved in fresh dry chloroform. The solution was passed down a column of magnesium oxide grade III (Nicholas, 1951), packed in chloroform, and the chromatogram developed with chloroform containing 0·5, 1·0 and 2·0% methanol successively. A brownish very red-fluorescent band formed rapidly and moved down the column on adding the 2·0% methanol in chloroform mixture. The dark brown non-fluorescent band at the top of the column did not move, and was discarded. The red-fluorescent band was collected, filtered, and the methanol washed out with water. This band gave a spectrum (Hartridge)

626·1 570·5 536·4 501·4 mμ (in $CHCl_3$).

No shift of the bands was observed on substituting authentic uroporphyrin I for the *Arion* pigment in the spectroscope. The spectrophotometer (Unicam) readings were:

626 570 536 501 mμ (in $CHCl_3$).

Fischer (1926) obtained a spectrum for uroporphyrin:

626·2 570·5 537·3 500·8 mμ (in $CHCl_3$).

No other bands were formed on the column.

Paper chromatography

Long paper. A sample of the *Arion* pigment ester was hydrolysed by allowing it to stand in contact with concentrated hydrochloric acid at room temperature overnight, and the acid was removed by standing solution over solid potassium hydroxide in a vacuum desiccator. The free porphyrin was dissolved in a little 2:6-lutidine and spotted on strips of Whatman No. 4 filter-paper 50 cm long and 4 cm wide in the apparatus devised by the writer (1953). Authentic uroporphyrin I, coproporphyrin I and mesoporphyrin IX were used as markers in both adjacent and mixed spots, and the chromatograms were run at 23° C with 2:6-lutidine:water (5:3) in an atmosphere of ammonia for 12 h. The *Arion* pigment formed one spot only corresponding with the one given by the uroporphyrin marker at R_F 0·15. The spot of 'pseudo-uroporphyrin' described by Falk, Dresel, Benson & Knight (1956) was not observed.

Separation of isomers. The esterified pigment was examined by the technique of Falk & Benson (1953) for the separation of uroporphyrins I and III. Uroporphyrin I was the only isomer detected, without trace of uroporphyrin III.

Decarboxylation

The *Arion* pigment was heated in solution in 1% (w/v) hydrochloric acid in a sealed tube for 3 h at 180° C, and the contents chromatographed by the technique of Chu, Green & Chu (1951) using coproporphyrins I and III and uroporphyrin I as markers. The *Arion* pigment gave one spot only corresponding with coproporphyrin I.

Melting-point

The *Arion* pigment ester was crystallized from chloroform-methanol, and recrystallized. The melting-point, determined in a Gallenkamp brass-bobbin apparatus was 292·2° C (uncorrected). The following melting-points for uroporphyrin I have been recorded:

293° C	Fischer & Orth, 1937, pp. 501–2
284° C	Granick & Gilder, 1947
290° C	Fischer, quoted by Carrié, 1936
293° C	Rimington & Miles, 1951

The evidence presented leads to the conclusion that the porphyrin of the integument of *Arion ater* is uroporphyrin I.

EXAMINATION OF OTHER COLOURED FORMS OF *ARION*

Specimens of the other coloured forms of *Arion* were collected, and the integuments examined in the same way for uroporphyrin. The amount of porphyrin in each individual was roughly estimated by direct visual comparison with the same volume of an extract from a black slug in a fluorimeter. The results were rather striking, in that the amount of porphyrin present was directly proportional to the amount of dark pigment in the integument; thus, in the brown animals, there was less porphyrin than in the black; in the red, less than in the brown, and so on, until in the pale grey integuments there was no porphyrin at all.

DISCUSSION

The identification of uroporphyrin I in the integument of *Arion ater* provides yet another example of the occurrence of this pigment in molluscs (Kennedy & Vevers, 1954, 1956). The associated black pigment, which appears to be melanin, is clearly present as a protection against the effects of light upon the animal, since uroporphyrin is known to cause photosensitivity (Fischer & Zerweck, 1924; Schreus & Carrié, 1931; Macgregor, Nicholas & Rimington, 1952), and in those individuals which have little or no associated pigment, there is no uroporphyrin in the integument. It seems also that the red, brown and orange pigments occurring in the integuments of the various coloured forms of *A. ater* are protective in this way, since, as the colour becomes paler, the amount of porphyrin decreases. Perhaps these red, brown and orange pigments are stages in the formation of melanin, each of which is sufficient to protect the animal according to the amount of uroporphyrin present. (The writer has not been able to examine the integument of *A. rufus*, in which the red pigment called 'rufine' by Dhéré & Baumeler (1928*b*) appears.)

Specimens of the land snail *Cepaea nemoralis*, which had brown and yellow or brown and pink striped shells with grey integuments, had no uroporphyrin at all in either the shell or the soft parts.

Kennedy & Vevers (1954) and Kennedy & Dales (1958) have discussed the occurrence of porphyrins in invertebrates, and the significance of associated pigments in protecting the animals against photosensitivity.

A further interesting point is provided by the sea-cucumber, *Holothuria forskali* Delle Chiaje, which has two pigments in the integument, a melanin and a yellow pigment with a very intense green fluorescence, visible even in daylight. The animal is black all over, except for the ventral surface, which is yellow, and if it is turned with the yellow ventrum towards the light, the animal immediately begins to turn back so that the yellow part is concealed. This suggests that the yellow pigment photosensitizes the animal, and that the black melanin protects that portion of the integument which is normally exposed to light. The yellow pigment may act as an orientating mechanism, as suggested by Crozier (1914). Grassé (1948, p. 91) wrote: 'La surface dorsale tout entière est photoréceptive; quand on fait tomber en un point quelconque une lumière ponctiforme, la peau se déprime à l'endroit touché; presque toutes les espèces ont un phototropisme négatif et fuient la lumière d'une fenêtre; le passage d'une ombre devant un animal épanoui le fait se contracter; il y a sans doute un rapport entre cette sensibilité à la lumière et la présence dans les téguments d'un pigment jaunâtre à belle fluorescence verte.' In some experiments carried out by the writer (unpublished) the pigment behaved in many ways like a flavin. The integument of *Cucumaria normani* Pace when exposed to light darkens and becomes black.

Kennedy & Dales (1958) have suggested that there is some parallel between the occurrence of free porphyrins in invertebrates and of these pigments in human porphyrias. In molluscs, if free uroporphyrin I is present, it occurs in the shell if there is one, or in the integument if there is not. The shell and the integument are both mantle products, and this is another example of the great affinity of uroporphyrin for tissues with a high calcium content.

SUMMARY

The porphyrin of the integument of *Arion ater* (L.) has been identified as uroporphyrin I. Chromatographic behaviour, reactions and melting-point are described. In the coloured forms of *A. ater*, the amount of porphyrin is shown to be directly proportional to that of the associated melanoid pigment. Reasons for this are suggested.

REFERENCES

CARRIÉ, C., 1936. *Die Porphyrine.* Leipzig: Georg Thieme.

CHU, T. C., GREEN, A. A. & CHU, E. J., 1951. Microchromatography of porphyrin esters. *J. biol. Chem.*, Vol. 190, pp. 643–6.

CROZIER, W. J., 1914. The orientation of a holothurian by light. *Amer. J. Physiol.*, Vol. 36, pp. 8–20.

DHÉRÉ, Ch. & BAUMELER, Ch., 1928*a*. Sur la porphyrine tégumentaire de l'*Arion empiricorum. C.R. Soc. Biol., Paris*, T. 99, pp. 726–8.

—— 1928*b*. Sur la rufine, pigment tégumentaire de l'*Arion rufus. C.R. Soc. Biol., Paris*, T. 99, pp. 492–6.

FALK, J. E. & BENSON, A., 1953. Separation of uroporphyrin esters I and III by paper chromatography. *Biochem. J.*, Vol. 55, pp. 101–4.

FALK, J. E., DRESEL, E. I. B., BENSON, A. & KNIGHT, B. C., 1956. Studies on the Biosynthesis of Blood Pigments: 4. The nature of the porphyrins formed on incubation of chicken erythrocyte preparations with glycine, δ-aminolaevulinic acid or porphobilinogen. *Biochem. J.*, Vol. 63, pp. 87–94.

FÉRUSSAC, J. B. L. d'A., BARON DE, FÉRUSSAC, A. E. J. P. J. F. d'A. DE & DESHAYES, G. P., 1819–51. *Histoire naturelle des Mollusques terrestres et fluviatiles.* 4 Tomes et Atlas. Paris.

FISCHER, H., 1926. *Abderhaldens Handbuch der biologischen Arbeitsmethoden*, Abt. 1, Tl. 2, p. 169; Abt. 4, Tl. 5, p. 536.

FISCHER, H. & ORTH, H., 1937. *Die Chemie des Pyrrols*, Bd. 2. Leipzig: Akad. Verlag.

FISCHER, H. & ZERWECK, W., 1924. Über den Harnfarbstoff. *Hoppe-Seyl. Z.*, Bd. 137, pp. 176–241.

GRANICK, S. & GILDER, H., 1947. Distribution, structure and properties of tetrapyrroles. *Advanc. Enzymol.*, Vol. 7, pp. 305–57.

GRASSÉ, P. P., 1948. *Traité de Zoologie*, T. 11. Paris: Masson et Cie.

HOPPE-SEYLER, F., 1871. *Medizin-chemische Untersuchungen.* Heft 1–4.

KENNEDY, G. Y., 1953. Partition paper chromatography of the porphyrins. *Scand. J. clin. Lab. Invest.*, Vol. 5, pp. 281–4.

KENNEDY, G. Y. & DALES, R. P., 1958. The function of the heart-body in Polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 15–31.

KENNEDY, G. Y. & VEVERS, H. G., 1953. The biology of *Asterias rubens* L. V. A porphyrin pigment in the integument. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 235–47.

KENNEDY, G. Y. & VEVERS, H. G., 1954. The occurrence of porphyrins in certain marine invertebrates. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 663–76.

—— 1956. Porphyrin pigments in the tectibranch mollusc *Akera bullata* O. F. Müller. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 35–9.

MACGREGOR, A. G., NICHOLAS, R. E. H. & RIMINGTON, C., 1952. Porphyria cutanea tarda. *Arch. intern. Med.*, Vol. 90, pp. 483–504.

MACMUNN, C. A., 1886. On the presence of haematoporphyrin in the integument of certain invertebrates. *J. Physiol.*, Vol. 7, pp. 240–52.

NICHOLAS, R. E. H., 1951. *Biochem. J.*, Vol. 48, pp. 309–13.

RIMINGTON, C. & MILES, P. A., 1951. A study of the porphyrins excreted in the urine by a case of congenital porphyria. *Biochem. J.*, Vol. 50, pp. 202–6.

SCHREUS, H. & CARRIÉ, C., 1931. Beobachtungen bei einem Fall von kongenitaler Porphyrie. *Dermat. Z.*, Bd. 62, pp. 347–62.

J. mar. biol. Ass. U.K. (1959), 38, 33–40
Printed in Great Britain

SOME OBSERVATIONS ON THE SCYPHOMEDUSA *ATOLLA*

By F. S. Russell, F.R.S.
The Plymouth Laboratory

(Text-figs. 1–3)

During the last four years collections of plankton from deep water have been made regularly on the cruises of R.V. 'Sarsia'. The opportunity has been taken to study the biology of the scyphomedusa *Atolla wyvillei*. It was hoped that with material collected from the same area at different times of the year it might be possible to gain some information on the growth and length of life of this medusa. The collections have been supplemented by a number of specimens taken on recent cruises of R.R.S. 'Discovery II', and a total of nearly 300 medusae have been examined. The collections were made with the 2 m stramin ring trawl and the Isaacs–Kidd pelagic trawl mostly from the Bay of Biscay.

On all specimens sufficiently well preserved the following observations were made: diameter of the umbrella in millimetres, including marginal lappets; number of marginal tentacles; and degree of development of the gonads. In assessing the development of the gonad the specimens were grouped into three categories: (1) those in which there were no gonads, or in which the gonads were just appearing as crescent-shaped growths in which the sex could not be distinguished; (2) those in which the gonads were obviously not mature, but in which the two sexes could be determined; and (3) males and females which appeared to be mature or, at any rate, in which the gonads were completely full. In mature females the gonads are always distinctly separated one from the other, but in mature males the gonads become folded and touch one another so as to form an almost continuous ring.

The results of relating size to maturity of 281 specimens are given in Fig. 1. It will be seen that there are two separate peaks of mature specimens, one between 10 and 20 mm and the other between 60 and 70 mm. I first thought that this bimodality might indicate two breeding periods, perhaps at yearly intervals. Further examination of the data showed, however, that the small and the large mature specimens corresponding to the two peaks occurred in all months of the year in which samples were collected, namely February, March, April, May, June, July and November. It was impossible, therefore, to gain any information on rate of growth from these observations. This in itself was disappointing, but it left the occurrence of the two peaks of maturity to be explained.

There was the possibility either that some specimens mature at an early size and die, or that after spawning they continue growth and, after recovery of the spent gonads, spawn a second time . In the latter event it might have been expected that the two peaks of maturity would not be so completely separated.

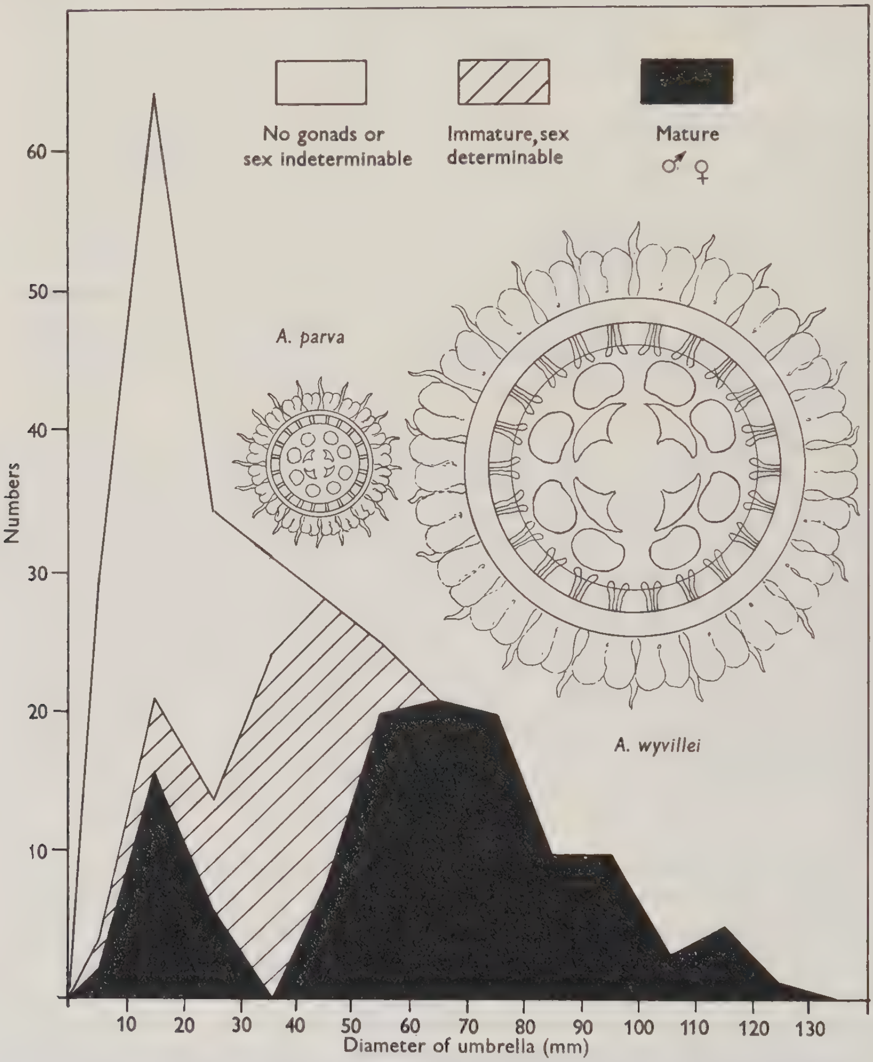


Fig. 1. Graph showing numbers of *Atolla* at different stages of maturity plotted against the diameter of the umbrella in millimetres. Diagrammatic outline drawings are given to show roughly the average natural size of mature female specimens of *A. parva* and *A. wyvillei*.

But when a comparison of the number of marginal tentacles in relation to the diameter of the umbrella was made a curious anomaly was noticed. Table 1 shows that the normal number of tentacles in full-grown medusae is 22, the number characteristic of *A. wyvillei*. Furthermore, this number was never exceeded in large specimens. There are, however, a few specimens less than 35 mm in diameter with as many as 24 tentacles. A special examination was therefore made of these specimens, and it was found that they were mature or already had gonads which were sexually distinguishable. Now, if these specimens were to continue growing it is most improbable that their marginal tentacles would be reduced in number, which alone would account for the complete absence of any larger specimens with more than 22 tentacles. If, on the other hand, these small medusae died after spawning, why should they grow more tentacles than the normal large adult?

The only reasonable explanation seemed that these medusae which mature at a small size are a different species from *A. wyvillei* which form the bulk of the collection.

Examination was made of the numbers of tentacles in all those specimens less than 35 mm in diameter with mature or sexually determinable gonads, or with more than 22 marginal tentacles. The results are given in Table 2, from which it can be seen that the number of tentacles ranges from 18 to 24; and that seventeen out of thirty-seven specimens have 20 tentacles, twelve have 24, and only one has 22.

All these specimens, and a number of others with 20 marginal tentacles, were then examined to see whether any morphological character could be found to distinguish them from *A. wyvillei*, other than size at maturity or number of tentacles.

A distinctive character which appears to be constant was found in the septa which separate the tentacular and rhopalar canals (Fig. 2). In *A. wyvillei* these septa tend to diverge widely towards the gastric sinus with their ends turning slightly inwards again. The thin part of the coronal muscle does not reach the ends of the septa so that there are always appreciable portions of the septa projecting centripetally beyond the muscle margin. In the medusae which mature at a small size the septa are nearly straight; some of them tend to have pointed ends and they only diverge slightly if at all near the gastric sinus. The thin portion of the coronal muscle covers the septa almost completely to their ends and some septa do not project at all centripetally beyond the muscle margin.[1] This continuation of the muscle ring to the ends of the septa emphasizes the points of entry of the tentacular and rhopalar canals into the gastric sinus and tends to increase the geometrical regularity of the colour pattern round the umbrella in this region (Fig. 3). The thin portion of the muscle itself also appears to be more strongly developed than in *A. wyvillei*. In some specimens, probably due to contraction, the ends of the septa extend farther than usual beyond the margins of the coronal muscle. These, however, can still be distinguished by the straightness of the septa themselves; usually also they have the typical number of 20 or 24 marginal tentacles.

---

[1] I have examined specimens of *A. vanhöffeni* and find that they also have this character.

TABLE 1. RELATION OF NUMBER OF MARGINAL TENTACLES TO DIAMETER OF UMBRELLA IN *ATOLLA*

Diameter (mm)	Number of marginal tentacles 17	18	19	20	21	22	23	24
1–9	.	.	.	5	.	6	.	1
10–19	.	2	3	19	5	28	1	5
20–29	.	.	1	7	.	27	.	5
30–39	.	.	.	3	2	23	.	1
40–49	1	.	2	3	4	18	.	.
50–59	.	.	1	1	1	23	.	.
60–69	1	.	.	3	2	17	.	.
70–79	.	.	.	4	2	10	.	.
80–89	.	1	1	.	1	15	.	.
90–99	.	.	.	1	.	8	.	.
100–109	.	.	.	.	.	4	.	.
110–119	.	.	.	.	.	3	.	.
> 120	.	.	.	.	1	4	.	.
Totals	2	3	8	46	18	186	1	12

TABLE 2. SPECIMENS OF *ATOLLA* LESS THAN 35 mm, INCLUDED IN TABLE 1, WITH MATURE OR SEXUALLY DETERMINABLE GONADS, OR 23 OR 24 TENTACLES

Diameter (mm)	Number of marginal tentacles 17	18	19	20	21	22	23	24
1–9	.	.	.	2	.	.	.	1
10–19	.	2	3	11	.	.	.	5
20–29	.	.	.	4	1	1	1	5
30–35	.	.	.	.	.	.	.	1
Totals	.	2	3	17	1	1	1	12

On the above characters I concluded that the small mature specimens were a distinct species and published a brief description under the name *Atolla parva* (Russell, 1958).

In the north-eastern Atlantic we thus have three species of *Atolla*: *A. wyvillei*, *A. vanhöffeni* and *A. parva*. There can, I think, be no doubt that the large medusae with 22 tentacles are *A. wyvillei* as they agree well with Haeckel's original description of the species based on five specimens 38–68 mm in diameter, all of which had 22 marginal tentacles except the smallest in which there were 19 (Haeckel, 1880, p. 488; 1881, p. 113). It is most unlikely that any of the existing specific names could be used for *A. parva*, although it must be present in other collections as it is generally stated that mature specimens are found at all sizes. Indeed, among the eight specimens on which Fewkes (1886, p. 939) based his description of *A. verrilli* there were two specimens 14 and 25 mm in diameter, each of which had 24 marginal tentacles.

As far as I have found at present *A. parva* differs from *A. wyvillei* only in its smaller size, the characteristic number of 20 or 24 marginal tentacles, and the form of the septa and their almost complete covering by the coronal muscle. The coloration of *A. parva* seems to be similar to that of *A. wyvillei*, and in some specimens the stomach alone is deeply pigmented.

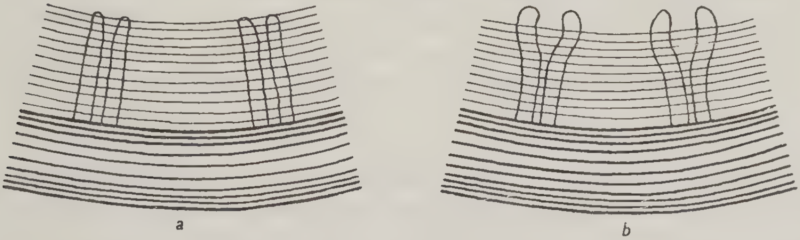


Fig. 2. Diagrammatic drawings to show the shapes of the septa separating the tentacular and rhopalar canals and their positions in relation to the thin portion of the coronal muscle: *a*, *Atolla parva*; *b*, *A. wyvillei*. Note that in *A. parva* the sides of the tentacular canals are approximately straight, and in *A. wyvillei* they are converging and constricted at the entrance to the gastric sinus.

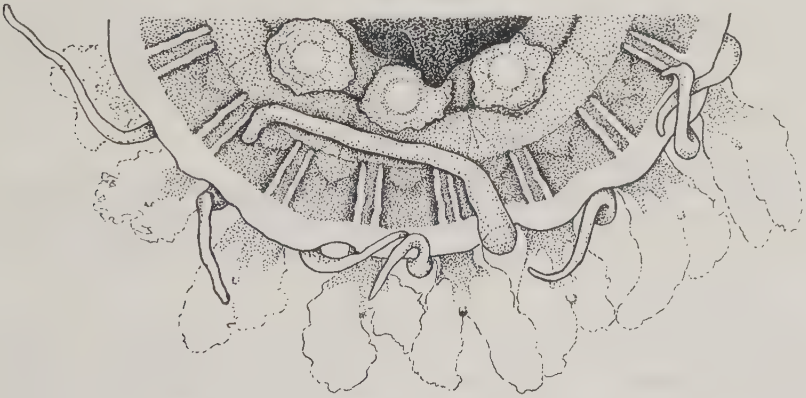


Fig. 3. Drawing of portion of the type specimen of *Atolla parva* to show the large marginal tentacle.

Since number of marginal tentacles is evidently a specific character the question naturally arises whether those specimens with 24 tentacles are specifically distinct from those with 20. I have examined the specimens carefully and can find no other character in which they differ, except that there is a tendency for those with 24 tentacles to be larger. I have now seen nearly fifty specimens: while the majority are between 9 and 21 mm in diameter there are four specimens which measure 25, 26, 29, and 30 mm in diameter respectively; each of these has 24 tentacles. Of these the largest, 30 mm in diameter (paratype B.M. 1958, 6.1.8), has only the stomach pigmented. As it cannot be said for certain that the medusae with 24 tentacles may not be a separate species I have selected as the holotype specimen of *A. parva* one with 20 tentacles.

From the data available there does not appear to be any difference in distribution between *A. wyvillei* and *A. parva* in the area from which the collections were made. The majority of the samples came from the Bay of Biscay as far south as the Spanish coast and along the continental edge west of the English Channel, the northernmost station being at 51° 28′ N., 12° 05′ W. Both species occurred over the whole area, and *A. parva* was also found at the following positions outside the Biscay area: 41° 11′ N., 14° 34′ W ('Discovery' Sta. 3374); 41° 26′ N., 09° 29′ W. ('Discovery' Sta. 3704); 40° 34′ N., 19° 42′ W. ('Discovery' Sta. 3661); and 36° 37′ N., 14° 09′ W. ('Discovery' Sta. 3700). All the collections were made in vertical or oblique hauls from considerable depths, but they were not sufficiently systematic to throw light on the vertical distribution of the medusae.

I have selected as the holotype a mature female specimen 18 mm in diameter. In this specimen the diameter to the periphery of the coronal muscle is about 13 mm; the diameter of the central umbrella disc is 7 mm and that of the base of the stomach 4·3 mm. It has 20 marginal tentacles, and the central disc of the umbrella has 19 notches. The specimen is well pigmented, and the gonads are pigmented on their exumbrellar surfaces and around their subumbrellar margins leaving the centre white. The colour is the typical brownish red. In each of the gonads there is a large egg about 0·75 mm in diameter with the smaller eggs round the periphery.

The specimen was caught in a 2 m stramin ring trawl from R.V. 'Sarsia' on 3 June 1957 at 45° 47′ N., 5° 00′ W. It has been deposited in the British Museum (Natural History) and has been given the registration number B.M. 1958. 6.1.1.[1]

While making these observations on *Atolla* it has been noticed that in some of the specimens one of the marginal tentacles is unusually large. I have not yet had time to make a study of this, but it is a noticeable feature in some small

---

[1] I have also deposited the following specimens as paratypes, B.M. 1958. 6.1.2–8:

13.vi.56. 48° 29′ N., 9° 5′ W. R.V. 'Sarsia'

2. 16 mm diameter	19 tentacles	Female
3. 26 mm diameter	24 tentacles	Immature
4. 7 mm diameter	20 tentacles	Immature

4.vii.56. 47° 02′ N., 5° 53′ W. R.V. 'Sarsia'

5. 20 mm diameter	20 tentacles	Male
6. 20 mm diameter	20 tentacles	Immature
7. 7 mm diameter	24 tentacles	Immature

29.ii.57. 41° 11′ N., 14° 34′ W. ('Discovery' Sta. 3374)

8. 30 mm diameter	24 tentacles	Female (stomach only pigmented)

specimens and is a further indication of the bilateral symmetry indicated by the number of notches or grooves in the central disc of the umbrella, which is usually one less than the number of marginal tentacles (Stiasny, 1934, p. 370). It will be necessary to get very young specimens to see whether this bilateral symmetry is a significant indication of the sequence of early development in the number of marginal tentacles.

The type specimen of *A. parva* has one such tentacle very much larger and more heavily built than the others (Fig. 3); and this is situated opposite the widest of the sectors of the central disc of the umbrella, that is at its point of asymmetry.

Luminescence has been observed in *A. parva* by Nicol (1958, p. 719).

My thanks are due to Captain C. A. Hoodless and the crew of R.V. 'Sarsia', the captain and crew of R.R.S. 'Discovery II', and a number of members of the staff of the Plymouth laboratory and the National Institute of Oceanography for their care in picking out some of the specimens from the catches.

SUMMARY

In a study of the biology of *Atolla* it has been found that at any time of year the population from the Bay of Biscay shows two peaks at maturity, one between 10 and 20 mm diameter and the other between 60 and 70 mm.

It is shown that the smaller of the two peaks is composed of specimens of *A. parva*, while the larger specimens are *A. wyvillei*.

REFERENCES

Fewkes, J. W., 1886. Report on the medusae collected by the U.S.F.C. steamer Albatross in the region of the Gulf Stream, in 1883–84. *Rep. U.S. Comm. Fish.*, Pt. 12 (1884), Appendix D, 36, pp. 927–77, Pls. 1–10.

Haeckel, E., 1880. *System der Acraspeden*, pp. 361–632. Jena.

—— 1881. Report on the deep-sea medusae. '*Challenger*' *Rep.*, Vol. 4, pp. i-cv, 1–154, Pls. 1–32.

Nicol, J. A. C., 1958. Observations on luminescence in pelagic animals. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 705–52.

Russell, F. S., 1958. A new species of *Atolla*. *Nature, Lond.*, Vol. 181, p. 1811.

Stiasny, G., 1934. Scyphomedusae. '*Discovery*' *Rep.*, Vol. 8, pp. 329–96, Pls. 14 and 15.

Note added in proof

Through the kindness of Dr P. L. Kramp I have been able to see additional specimens which were picked out by Dr Kay Petersen from the collections of *Atolla* from the Atlantic in the Universitetets Zoologiske Museum in Copenhagen. Many of these were in rather poor condition with gonads missing; but the fact that none of these specimens had 22 tentacles confirms their identification. Of these eight had 20 tentacles, eight had 24, and four had 26, the highest number so far seen. It is interesting to note that the four specimens with 26 tentacles came from the farthest north stations in the Norwegian Sea, and that one of these was 33 mm in diameter.

			Metres wire	Diameter (mm)	Sex	No. of tentacles
Ingolf						
20. vii. 1896	St. 112	67° 56′ N., 6° 44′ W.	—	15	♀	26
				22	—	26
				23	—	24
				25	—	26
Tjalfe						
3. v. 1909	St. 322	60° 07′ N., 48° 26′ W.	2000	22	—	24
8. v. 1909	St. 336	64° 06′ N., 55° 18′ W.	1040 –1100	*c.* 13 (damaged)		
				14	—	24
Thor						
28. ii. 1909	St. 68	36° 13′ N., 9° 44′ W.	3000	13	—	20
				13	—	20
				16	—	24
				20	—	20
4. iii. 1909	St. 71	39° 35′ N., 9° 45′ W.	1600	11	—	24
				13	♀	24
9. ix. 1910	St. 232	36° 28′ N., 9° 06′ W.	2000	7	—	? 20
				10 (shrunk)	♀	24
				15	—	24
Dana						
24. v. 1934	St. 5143	65° 14′ N., 6° 06′ W.	2400	33	♂	26
Atlantide						
29. i. 1946	St. 82	5° 27′ N., 0° 07′ E.	1700	8	—	20
2. iv. 1946	St. 139	1° 30′ N., 10° 10′ W.	1750	10	—	20
				10	—	20
				12	—	20
				25 (damaged)		

J. mar. biol. Ass. U.K. (1959) 38, 41–45
Printed in Great Britain

CHEMICAL CHANGES IN SEA WATER OFF PLYMOUTH DURING 1957

By F. A. J. Armstrong and E. I. Butler
The Plymouth Laboratory

(Text-figs. 1–3)

Analyses of sea water collected during 1957 at the International Hydrographic Station E1 (lat. 50° 02′ N., long 4° 22′ W.) are given here in the same form as in earlier reports (Armstrong, 1954, 1955, 1957, 1958). The methods of collection and of analysis for phosphorus and silicon are substantially unchanged. Some analyses were made for ammonia by a vacuum distillation method (Riley, 1953), and for inorganic nitrogen (nitrate + nitrite + ammonia) by reduction of nitrate and nitrite with nickel (Riley & Sinhaseni, 1957) to ammonia, which was vacuum distilled. Salinities were determined by the Government Chemist's Department.

We wish to express our thanks to Lt.-Cdr. C. A. Hoodless and the crew of R.V. 'Sarsia' and to Capt. W. J. Creese and the crew of R.V. 'Sula', for assistance at sea.

RESULTS

Temperature and salinity

The vertical distribution of temperature during the year is shown in Fig. 1. The lowest surface temperature recorded was 9·7° C. on 15 February, and the highest was 16·11° C. on 16 July. Some vertical irregularities of temperature and salinity occurred in March and April, being most marked on 24 April, when water of salinity higher than on 11 April, higher than in the layers above, and marked by its lower silicate content, was present at 50 and 70 cm. (Table 2). As is seen there was a temperature minimum at 20 m, but the densities show the vertical stability.

A sharp thermocline between 15 and 20 m was established by 11 June, and persisted with some change of level until September. From October until the end of the year the water column was isothermal.

Phosphate

Vertical distribution is shown in Fig. 2, and integral mean concentrations in Table 1. The winter maximum found was 0·47 μg atom P/l. on 15 February, and the lowest concentrations were 0·08–0·09 μg atom P/l. at the 5 and 10 m levels in May and June. Phosphate remained low in the upper 20 m until September; the vertical distribution had become uniform by 15 October, and so remained until the end of the year.

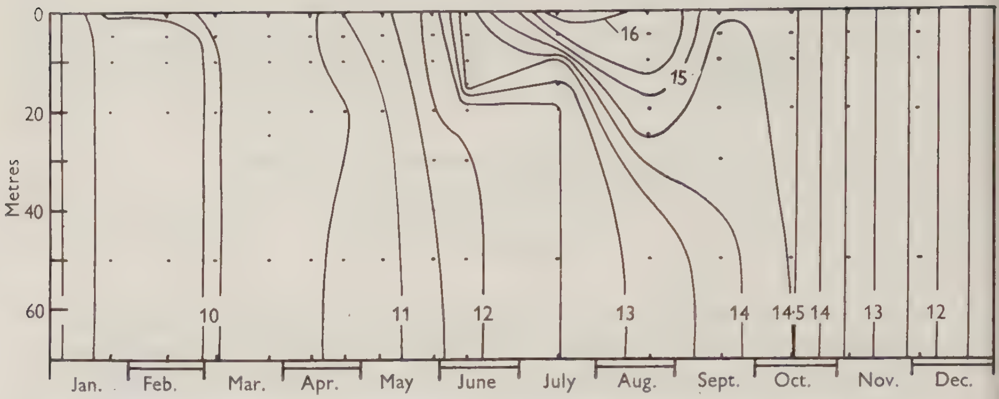


Fig. 1. Vertical temperature distribution at International Hydrographic Station E1, 1957. Contour lines at 0·5° C intervals.

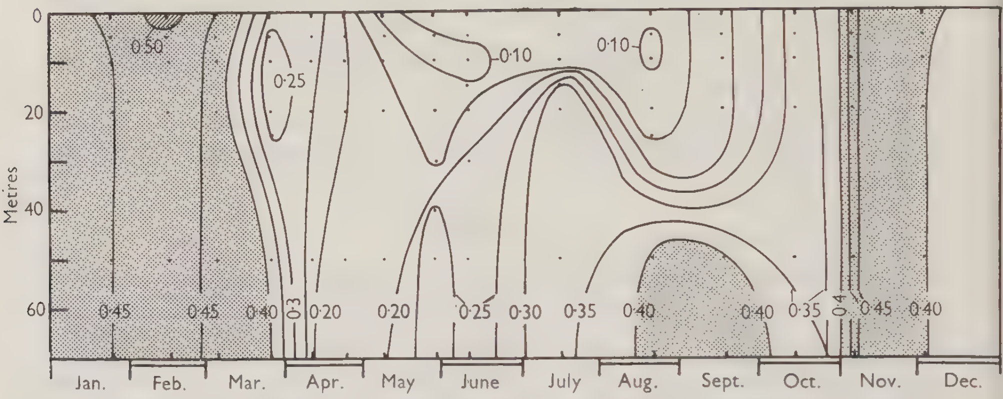


Fig. 2. Vertical distribution of phosphate at International Hydrographic Station E1, 1957. Contour lines at 0·05 μg atom P/l. intervals.

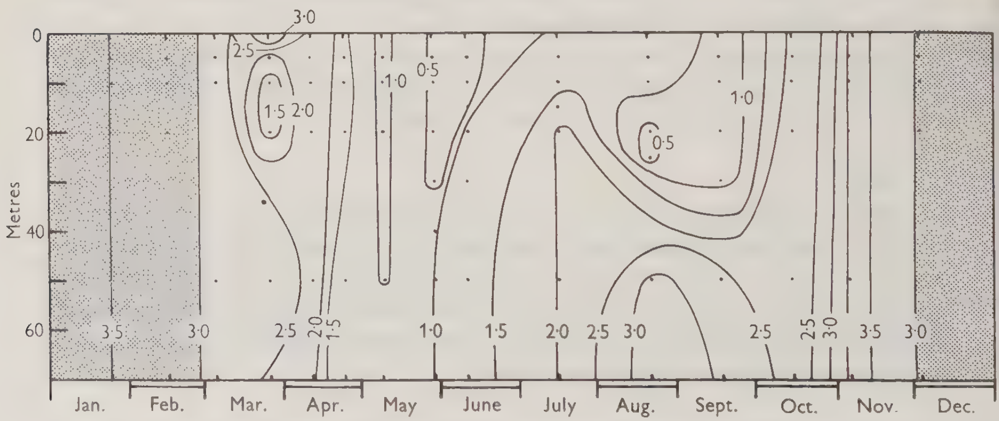


Fig. 3. Vertical distribution of silicate at International Hydrographic Station E1, 1957. Contour lines at 0·5 μg atom Si/l. intervals.

'Total phosphorus'

Determinations were made in January, February and March only, to find the winter maximum value. This was 0·63 μg atom P/l. on 15 February.

Silicate

Vertical distribution is shown in Fig. 3, and integral mean concentrations in Table 1. The maximum found at the beginning of the year was 3·5 μg atom Si/l. on 24 January. On the whole, higher silicon concentrations than usual were maintained during the year, as Table 1 shows. Values of less than 0·5 μg atom Si/l. were found only on 29 May and 11 June, above the thermocline, and, surprisingly, at 20 and 25 m on 21 August. These low values came between considerably higher ones in the water above and below, as shown in Table 3.

TABLE 1. INTEGRAL MEAN CONCENTRATIONS IN WATER COLUMN AT STATION E1, 1957

Date	Phosphate (μg atom P/l.)	'Total-P' (μg atom P/l.)	Silicate (μg atom Si/l.)	Ammonia (μg atom N/l.)	Inorganic N (μg atom N/l.)
24 Jan.	0·45	0·52	3·47	0·7	9·8
15 Feb.	0·47	0·63	3·35	—	9·6
5 Mar.	0·43	0·54	2·71	—	—
26 Mar.	0·31	—	2·30	0·7	1·7
11 Apr.	0·24	—	2·25	—	—
24 Apr.	0·21	—	1·22	—	—
9 May	0·16	—	1·07	—	—
29 May	0·20	—	0·72	1·1	2·6
11 June	0·18	—	1·03	0·7	1·6
16 July	0·30	—	1·83	—	—
21 Aug.	0·26	—	1·87	—	—
17 Sept.	0·31	—	1·62	1·2	6·9
15 Oct.	0·31	—	2·28	1·2	—
6 Nov.	0·44	—	3·58	—	—
3 Dec.	0·42	—	3·07	0·4	3·7

There was a significant fall in salinity in the upper 10 m between July and August. This may show that water at this level has been replaced by other of different chemical properties, for although phosphate fell, silicate rose slightly, at the same time. There were also slight changes in the composition of the bottom water, but the change of salinity here may not be a significant one, and the changes in phosphate and silicate could be put down to a normal regeneration of these nutrients. The data are insufficient to explain the curious vertical distribution of silicate.

Layering of water masses at this station (apart from the annually developed thermocline) has been found from time to time in the past 8 years, having been revealed by irregularities in the silicate figures. Occasionally, laminar as well as bodily displacements of water must occur at E 1.

TABLE 2. OBSERVATIONS AT STATION E1, 24 APRIL 1957

Depth (m)	Temperature (° C)	Salinity (‰)	Density *in situ*	Phosphate (μg atom P/l.)	Silicate (μg atom Si/l.)
0	11·0	35·19	26·94	0·20	1·5
5	10·88	35·13	26·91	0·19	1·5
10	10·83	35·14	26·93	0·20	1·4
20	10·45	35·16	27·01	0·21	1·8
50	10·73	35·29	27·06	0·17	0·8
70	10·65	35·30	27·09	0·25	0·8

TABLE 3. OBSERVATIONS AT STATION E1, 16 JULY AND 21 AUGUST 1957

Depth (m)	Salinity (‰)		Phosphate (μg atom P/l.)		Silicate (μg atom Si/l.)	
	July	Aug.	July	Aug.	July	Aug.
0·5	35·13	35·12	0·11	0·11	1·2	1·6
5	35·16	35·10	0·15	0·09	1·3	1·4
10	35·17	35·08	0·14	0·10	1·3	1·4
15	35·18	—	0·36	—	1·7	—
20	35·20	35·16	0·34	0·12	2·0	0·2
25	—	35·16	—	0·14	—	0·3
50	35·20	35·17	0·33	0·43	2·0	3·2
70	35·21	35·18	0·34	0·42	2·0	3·3

Nitrogen

Enough analyses to draw an isopleth could not be done, and integral mean values only are given here. The vertical distribution did, however, resemble that for phosphate. The values (Table 1) in winter and summer resemble those obtained at E1 by other methods in earlier years (Cooper, 1933). The winter (maximum) ratio, N/P = 20/1 by atoms or 9·2/1 by weight, was a little higher than the 16 or 17/1 atomic ratio found in 1931 by Cooper.

Integral mean concentrations

The spring decreases representing consumption of nutrients were: phosphate 0·29 μg atom P/l., silicate 2·75 μg atom Si/l., inorganic nitrogen 8·0 μg atom N/l. The ratio N/P consumed was 27·6/1 μg atoms or 12·4/1 by weight and is rather higher than the mean ratio, 16·3/1, for a number of analyses of plankton from this area (Cooper, 1937).

SUMMARY

The results of analysis of sea-water samples from the International Hydrographic Station E1 during 1957 are given in graphical form and as integral mean values for the water column of 70 m. The seasonal variation shows the consumption of nutrients during the spring growth of plants to have been: phosphate 0·29 μg atom P/l., silicate 2·75 μg atom Si/l., inorganic nitrogen 8·0 μg atom N/l. At the time of winter maximum the N/P ratio was 20/1 by

atoms or 9·2/1 by weight. The ratio of these elements consumed was 27·6/1 by atoms or 12·4/1 by weight.

Unusual vertical distributions of silicate were found in April and August, and are attributed to laminar water movements.

REFERENCES

ARMSTRONG, F. A. J., 1954. Phosphorus and silicon in sea water off Plymouth during the years 1950 to 1953. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 381–92.

—— 1955. Phosphorus and silicon in sea water off Plymouth during 1954. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 223–28.

—— 1957. Phosphorus and silicon in sea water off Plymouth during 1955. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 317–19.

—— 1958. Phosphorus and silicon in sea water off Plymouth during 1956. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 371–77.

COOPER, L. H. N., 1933. Chemical constituents of biological importance in the English Channel, November 1930 to January 1932. Part I. Phosphate, silicate, nitrate, nitrite, ammonia. *J. mar. biol. Ass. U.K.*, Vol. 18, pp. 677–728.

—— 1937. On the ratio of nitrogen to phosphorus in the sea. *J. mar. biol. Ass. U.K.*, Vol. 22, pp. 177–82.

RILEY, J. P., 1953. The spectrophotometric determination of ammonia in natural waters, with particular reference to sea water. *Anal. chim. acta*, Vol. 9, pp. 575–89.

RILEY, J. P. & SINHASENI, P., 1957. The determination of ammonia and total ionic inorganic nitrogen in sea water. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 161–68.

J. mar. biol. Ass. U.K. (1959), 38, 47–56
Printed in Great Britain

THE GROWTH AND FRUITING OF *GRACILARIA VERRUCOSA* (HUDSON) PAPENFUSS

By W. Eifion Jones

Marine Biology Station, Menai Bridge, Anglesey

(Text figs. 1–3)

Several workers have observed, in Europe and elsewhere, that *Gracilaria verrucosa* is a more conspicuous component of the flora in the summer months (Cotton, 1912, p. 134; Rosenvinge, 1931, pp. 602–6; Gibb, 1939, p. 378; Causey, Prytherch, McCaskill, Humm & Wolf, 1946; May, 1948), with the natural inference that its growth rate is greater in that season. However, the quantity present on the shore at any time depends also on the rate of defoliation by the waves. This will be higher in winter. Observation under more controlled conditions was therefore considered desirable.

Fragmentary records of the fruiting of *G. verrucosa* extend over many years. Hudson (1762, pp. 588, 591) gave the fruiting period (for female gametophytes) as being from June to September. Greville (1830, p. 123) agreed but added that occasional fruiting plants could be found at all seasons. Feldmann (1954, p. 86) recorded, in Brittany, cystocarps in January, April, July, August, September, October and December and tetraspores in August and September. Rosenvinge (1931, pp. 602–6) recorded, in Denmark, ripe cystocarps in July and August; empty ones in October and April; ripe tetrasporangia in June, July and August and empty ones in October; spermatangia in July and August. The present attempt to obtain a more complete picture has involved the examination of collections of plants from various shores at all times of the year; and estimation of the relative numbers of cystocarpic and non-cystocarpic plants on a shore. These field observations have been supplemented by testing plants in the laboratory for their readiness to shed spores and the capacity of the latter to germinate.

THE VEGETATIVE GROWTH CYCLE

Criterion of growth

The increasing length of a frond of *Gracilaria* probably furnishes a satisfactory measure of its growth since, in a uniformly cylindrical thallus, the bulk is proportional to the length. However, the simple measurement of the maximum length of the thallus did not appear to be satisfactory, since the growth of the more or less numerous branches would not necessarily be represented by this. Instead, in the present investigation, the fresh weight of the plants concerned was periodically measured.

Methods

In their experiments on factors affecting growth in *Gracilaria verrucosa* in N. Carolina, Causey *et al.* (1946) measured the 'green weight' of the plants after shaking off the surface water. As the plants used in the present investigation were not large, incomplete removal of surface water was thought likely to cause inaccuracies, as was the inevitable loss of weight by desiccation during weighing; in addition the careful blotting of plants is very time-consuming. To avoid this, weighing was carried out with the plants completely immersed in sea water, as described elsewhere (Jones, 1959, p. 155).

The material used consisted of plants or parts of plants divided at the holdfast and attached by nylon fishing 'gut' to 'Tufnol' plastic bars. The bars were mounted in a box with sides, top and bottom of half-inch mesh wire-netting which allowed a free flow of water round the plants while preventing the entry of floating debris. The box was suspended in one compartment of an open-bottomed barge anchored in the Menai Straits. The bars, with the attached plants, were brought into the laboratory periodically for inspection.

Numerous algal spores settled on the plants while on the barge and a heavy growth of epiphytes resulted in which silt was often bound. This reduced the amount of light reaching the plant. In addition some shade was given by the sides of the barge and, occasionally, by masses of floating weed (*Ascophyllum nodosum* and *Fucus vesiculosus*) which entered the barge and had to be removed. These conditions did not appear to be ideal for the growth of the plants which, during the first season, did not survive indefinitely in an actively growing state. In the subsequent season a more continuous record was obtained. Epiphytes, which had to be removed laboriously by hand, were a major difficulty in the later stages of growth, particularly in the second season.

Results

In the first season batches of six plants were used which were replaced by freshly collected batches when signs of mortification appeared or when the growth of epiphytes became particularly heavy. Results were not obtained for all months: the general pattern was that a comparatively low growth rate of 1 % increase in fresh weight per day was observed in November, December, January and April and that in May a sudden increase in the growth rate to 2·6 % per day occurred. This rate was maintained through June and gradually declined through the summer, falling more rapidly with the approach of autumn.

These experiments also suggested that the growth rates of cystocarpic and tetrasporic plants were not always the same. In the second season, therefore, the growth rates of three cystocarpic and three tetrasporic plants were recorded separately. In this series the changes in weight of the same individuals were followed from September until the following July. Deterioration of the plants, resulting mainly from their having grown so large as to be damaged by abrasion against the sides of the box, then made it necessary to conclude the experiment. Fig. 1 shows the changes in the total weight of the three tetrasporic and three cystocarpic plants. In general these results confirm those of the previous season, showing a tendency for the weight of the plants to decline through the winter with a slight rise in early spring followed by a rapid increase in May. Examined separately, however, the results for tetrasporic and cystocarpic plants show some differences.

(i) The spring burst of growth began about a fortnight later in cystocarpic than in tetrasporic plants. A parallel increase in weight was shown by two additional batches, of three tetrasporic and three cystocarpic plants respectively, collected in late April and grown in the box together with the original plants during the period of the May increase. These results are included in Fig. 1 and offer some confirmation of the observations, the parallel being particularly close in the cystocarpic plants.

(ii) From September onwards, while the weight of the tetrasporic plants rose steadily, that of the cystocarpic decreased at almost the same rate. Occasional increases in the weight of the cystocarpic plants (as between 13 and 25 Oct. in Fig. 1) suggest that their potential growth rate at that time of the year is as high as that of tetrasporic plants. Their loss of weight seems to result from more rapid decay of the thallus. It has been observed that decay in the autumn is more pronounced in cystocarpic plants, apparently because pathogens find easy entry into their thalli through the ostioles of cystocarps after spore shedding has ceased. Very frequently decay may be seen to be beginning in a cystocarp while the thallus remains healthy above and below it. Decay soon spreads to the thallus itself and that part above the diseased area breaks off and is lost. In tetrasporic plants decay is largely limited to damaged tips, spreading slowly down the branches and causing comparatively slow loss of material. Probably pathogenic organisms do not enter as easily into the comparatively small opening left after the discharge of the tetraspores, while the copious mucilage left in the cystocarp, and often exuded through the ostiole as fruiting finishes, offers a good substrate for the settlement and development of the spores of pathogens.

In the sheltered conditions of the raft a steady state was reached by the end of November in tetrasporic plants and by January in cystocarpic, during which loss and growth were roughly balanced and little change in weight occurred until the rapid spring growth commenced.

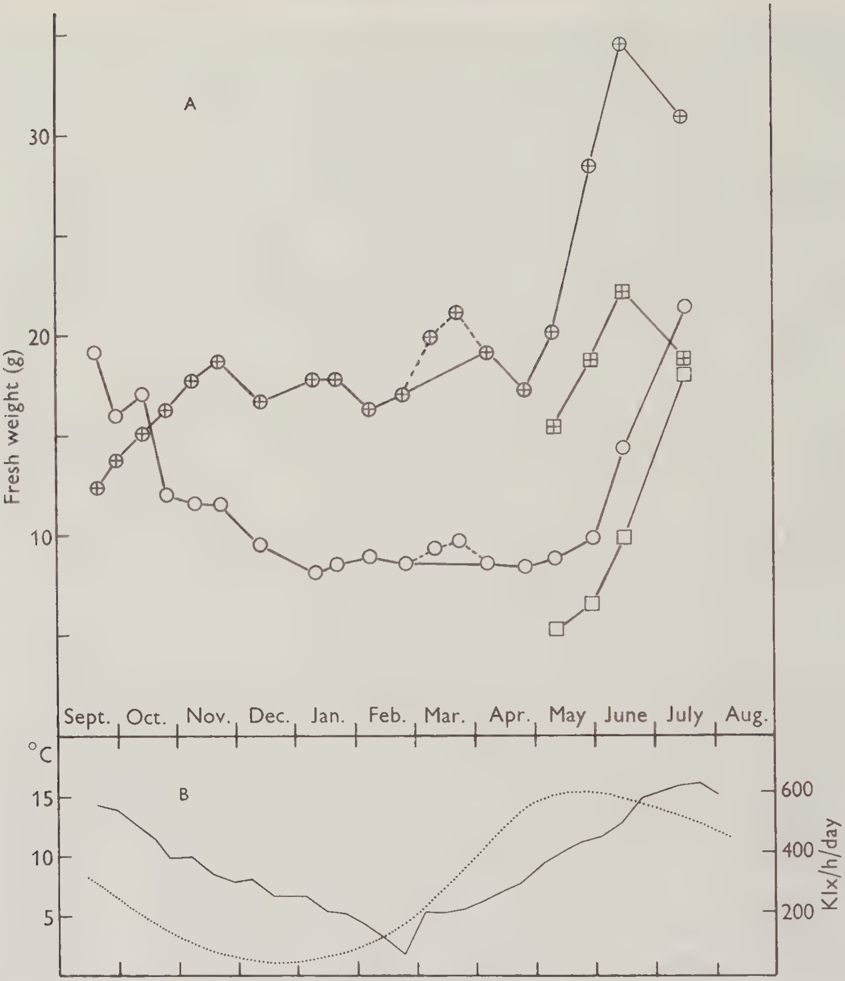


Fig. 1. A. Changes in the total weight of four groups of three plants of *Gracilaria verrucosa* grown on a barge in the Menai Straits. ⊕, tetrasporic, ○, cystocarpic, grown from September 1955; ⊞, tetrasporic, □, cystocarpic, grown from May 1956. The broken line in the period February–March shows the growth recorded without removing epiphytes which were removed at the beginning of April. The solid line gives the more correct picture of the growth of *Gracilaria*. B. Continuous line, sea surface temperatures (10-day averages) over the same period, measured at Menai Bridge Pier. Dotted line, average illumination for Plymouth, 1930–37 (from Atkins, 1938, and Harvey, 1955).

Discussion

In these experiments the plants, though growing in the open, were not under exactly the same conditions as in a normal littoral habitat. In particular:

(i) There was no tidal effect, i.e. no emersion, no pressure fluctuation and no alteration of light intensity by absorption by varying depths of water.

(ii) Although movement of the barge caused plants to sweep against the sides of the box, there was no effect comparable to wave action as on a beach.

(iii) Owing to shading by the sides of the barge the light intensity in the box was never as high as the maximum obtainable on the shore at the same season.

(iv) Temperature changes in the Straits are more pronounced than in the open sea. There may, for instance, be rather sudden falls of temperature in February to as little as 2° C.

The effect of these differences, with the exception of the last, should be to even out the seasonal changes in weight so that, while the experiments indicate the general trend, more pronounced changes might be expected on the shore. This is particularly true of shelter from wave action; the loss resulting from wave damage is severe in winter on the shore and certainly greater than that shown in the experimental results. The equilibrium between the growth rate and the rate of loss by decay and damage, mentioned above, is not so readily established on the shore where plants decrease in size throughout the winter and early spring.

The results show an increase in the weight of both tetrasporic and cystocarpic plants in March. This may be attributed, in the main, to the growth of epiphytes which became noticeable at the end of February. Some real increase in weight did, in fact, occur during this period but was small, as shown by the values for 25 April when careful cleaning of the plants, with the removal of all epiphytes, was commenced. The spring burst of growth began about 3 weeks after this (in the tetrasporic plants) so that it seems that *Gracilaria* begins active growth later than some other species.

In considering the relationship of the seasonal environmental changes to the growth cycle the two factors with obvious summer maxima are the sea temperature and the daily quantity of light. Conversely, the acceleration of growth occurs when the supply of dissolved nitrogen and phosphorus in the sea water is approaching its lowest annual value (Harvey, 1955, pp. 44–59). In Fig. 1 the annual cycles of temperature and light are shown on the same time scale as the growth cycles of tetrasporic and cystocarpic plants over the period September 1955–July 1956. It will be seen that, whilst growth broadly follows the trend of both factors, there is no exact coincidence. For instance, the burst of growth in May does not coincide with a sudden increase in either temperature or light, which have both been increasing for several months before this. Certain correlations do, however, appear reasonable, though hypothetical at the moment.

Accepting the loss of weight of cystocarpic plants in autumn as being due to decay, the potential growth rate may best be seen in the tetrasporic plants. In these growth continued until late November, when, as has been noted above, it decreases below that required to maintain the weight of the plant. At this time the falling temperature of the sea reached about 8° C. Until the end of April, when the growth rate again overtook the rate of loss, the temperature remained below 8° C, suggesting that, under the conditions on the barge, this represents a critical temperature below which the level of metabolism of the plants is too low to replace all the material lost by accidental damage. The amounts of light available at these times are, on the other hand, very different. At the end of November the daily quantity of light is approaching its minimum value, while in late April it has reached about 85% of its maximum. This critical temperature hypothesis receives some support from the fact that a slight rise in the growth rate may be seen coincident with the rise in temperature after the February minimum; this is small and not necessarily significant but appears to occur in both tetrasporic and cystocarpic plants. Again, the results of Causey *et al.* (1946) are in general agreement with this hypothesis; from their observations on *Gracilaria* in N. Carolina they state that growth begins in spring when the water temperature rises above 10° C and ceases when it drops below 10° C in the autumn. It should be remembered, however, that although some growth occurred in the cystocarpic plants at the same time as the tetrasporic, the main burst of growth began a fortnight later, by which time the temperature had risen to nearly 10° C.

This time lag may perhaps be explained by analogy with the behaviour of unicellular algae in culture. Spencer (1954) showed that when, after a period of growth in suboptimal conditions, the plants are subcultured to media containing ample nutrients, there is a time lag before rapid growth recommences. The duration of this lag is related to the length of the period of suboptimal nutrition. Something similar may be taking place in the present instance. Cystocarpic plants suffer greater loss by winter decay and damage than tetrasporic plants and may have larger deficiencies to make good in spring before rapid growth can begin.

When the necessary experimental facilities become available it is hoped that these questions will be more completely investigated.

Ecological significance of the results

The results described above are in agreement with the cycle of events observed on the shore, where plants reach their maximum size in late summer, suffer considerable defoliation in autumn and continue to be reduced by damage through the winter. The fact that growth continues to some extent through the winter, although it is usually outweighed by damage, is of significance in determining some of the variations in form of plants in exposed and sheltered habitats, which will be described in another paper.

THE REPRODUCTIVE CYCLES

Tetraspores

Tetraspore production begins early in the year. Plants may be found in February from the older parts of which almost all tetraspores have been shed but whose younger branches contain immature sporangia. Such plants have been found mainly in the more sheltered localities (such as Porth Penrhyn Mawr, within the Holyhead breakwater). Elsewhere there is little survival of the parts which bore tetraspores in the previous season and the tetrasporic plants found in February contain only immature sporangia. These are generally located in the distal parts of the branches, which are swollen to about twice the diameter of the sterile parts. This is a point of some taxonomic interest (Jones, 1957). No spores are shed naturally from plants brought into the laboratory at this stage. In March mature tetraspores can be found which, in the latter part of the month, are shed (though not as readily as later in the year) and begin division normally. Mature, viable tetraspores are produced continuously in the succeeding spring and summer months, the quantity increasing with the enlargement of the thalli. The maximum production appears to occur in July.

As autumn approaches an increasing number of plants is to be found which have shed most of their tetraspores. Such plants are common in August and, by October, the majority of the spores have been shed. As the loss by wave damage increases, less and less of the summer's tetrasporangial branches are to be found. However, as has been shown, vegetative growth continues into the autumn and early winter and, in the young branches resulting from this growth, some tetraspore production continues. Thus in November and December mature tetraspores may be found in the younger branches where their development causes a local swelling of the distal part of the branches similar to that seen in the early part of the year. Natural shedding and subsequent development of tetraspores has been observed in November from the young branches but the older parts of the thalli which produced spores in the summer do not readily shed any remaining spores after September. The tetraspores produced in the autumn branches form only a small proportion of the total crop. Fig. 2 summarizes the cycle of tetraspore production.

Carpospores

Female gametophytes bearing cystocarps may be found at all times of the year but the proportion of the total population which they constitute varies seasonally. Fig. 3 shows the percentage of plants bearing obvious cystocarps on the boulder beach at Dinas Dinlle. In recording these results the plants were not, as a rule, collected since the removal of samples of 100–150 plants on each visit would quickly destroy the population. Thus no differentiation of tetrasporic and male material was possible and, also, some underestimation of the total number of female plants might be expected in winter, when some plants bore only a few cystocarps which might be overlooked. This also occurred during the occasional rises in the sand level, particularly in the winter when, in general, the female gametophytes have suffered greater decay and damage and, being therefore smaller than the tetrasporophytes, are more likely to be obscured by the sand, as in the case of the first December value shown in Fig. 3. Nevertheless, the general trend is quite clear: plants bearing cystocarps are scarcest in April; their number increases in May and rises rapidly through the summer, reaching a maximum in autumn. The number remains high until January when a rapid fall begins and continues until the minimum is again reached. Except for a period in spring viable carpospores can be obtained at all times.

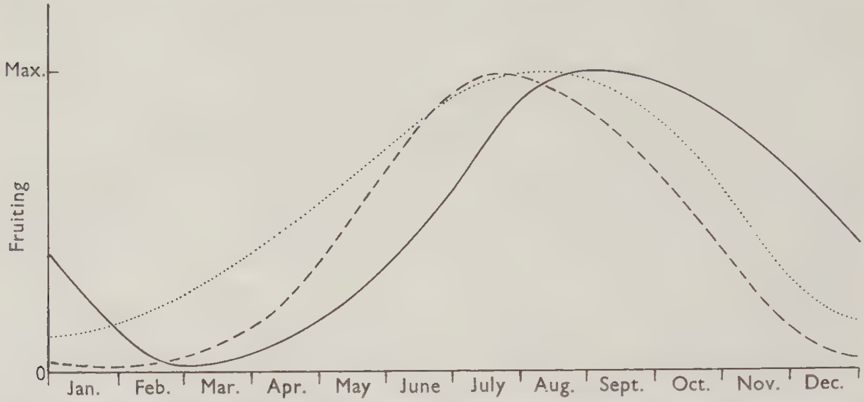


Fig. 2. Diagram of fruiting cycle in *Gracilaria verrucosa* indicating seasonal production of spores and spermatia. —, carpospores; - - -, tetraspores; ......, spermatia.

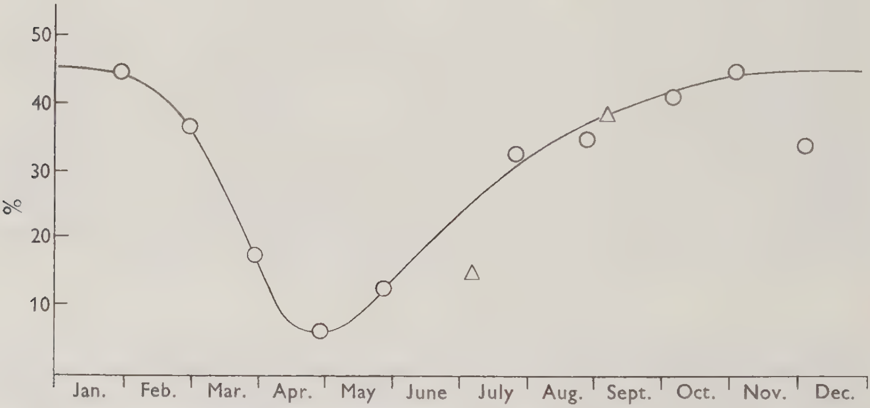


Fig. 3. Percentage of female gametophytes bearing cystocarps in a population of *Gracilaria verrucosa* at Dinas Dinlle. △, 1955; ○, 1956.

It should be pointed out that, although the maximum number of plants bearing cystocarps is found towards the end of November, this is not necessarily the time either of the largest total number of cystocarps or the maximum carpospore production. In fact the plants, in all but the most sheltered places, are considerably reduced in size by this time and the amount of frondage capable of bearing cystocarps is very much smaller than in July and August. Since the loss by decay and damage generally begins to be noticeable in late August and September, the maximum production of carpospores apparently occurs just before this (Fig. 2).

Comparison of the curves in Figs. 1 and 3 shows that there is a close correlation between the number of plants bearing cystocarps on the shore and the vegetative growth cycle. This is to be expected, for the severe loss of frondage that occurs in late winter and early spring often leaves only the basal parts of the plants and these are not usually fertile. Thus the appearance of cystocarps must await the growth of the new season's branches.

Spermatia

Male plants are very much less common than tetrasporic or female plants, and have not been found in most of the collections made. For this reason the cycle of spermatium production is not as clear.

Male plants have been observed in January, February, March, May and November, while in June and September, when the largest numbers were seen, the active emission of spermatia has been witnessed. The observations suggest a summer maximum of spermatium production, occurring before the carpospore maximum (presumably coinciding with the most active production of mature carpogonia) and a winter minimum, probably in December (Fig. 2).

Discussion

As may be seen from the foregoing, the vegetative growth and fruiting cycles are closely related. The most active production of tetraspores and spermatia takes place during the period of fastest growth, when the bulk of plant material is reaching its maximum. The maximum of carpospore production appears to be later than the others, probably because the production of mature carpospores from a carpogonium, which involves fertilization and the production of a massive gonimoblast 'tissue', requires more time than the development of mature tetraspores or spermatangial sori from a modified cortical cell.

Thus numerous large plants producing immense numbers of tetraspores and carpospores are present on the shore in June, July and August. During this period the calmest weather may be expected in most years in the N. Atlantic (Bigelow & Edmondson, 1947) and conditions for spore settlement should be most favourable.

This work formed part of an autecological study of *G. verrucosa*, carried out at the suggestion of Prof. L. Newton, whose advice and encouragement I am pleased to acknowledge.

SUMMARY

The annual cycles of vegetative growth and reproduction of *Gracilaria verrucosa* are described. The former has been investigated by growing plants on a barge in the Menai Straits. Growth occurs throughout the year, being slow in winter and suddenly increasing in May–June to a higher level. It is suggested that the temperature of the sea water is the most important factor in controlling the growth rate.

The study of the reproductive cycles shows that spore production continues throughout the year, except for a short period in early spring (February–March). The maximum production is in summer, tetraspores in July and carpospores in August–September. Spore production can be closely related to the vegetative growth cycle.

REFERENCES

Atkins, W. R. G., 1938. Photoelectric measurements of the seasonal variation in daylight. *Proc. roy. Soc.*, Ser. A, Vol. 165, p. 453.

Bigelow, H. B. & Edmondson, W. T., 1947. Wind waves at sea, breakers and surf. *Hydrographic Office U.S. Navy Publ.*, No. 602, pp. 72–80. Washington D.C.

Causey, N. B., Prytherch, J. P., McCaskill, J., Humm, H. J. & Wolf, F. A., 1946 Influence of environmental factors upon the growth of *Gracilaria confervoides*. *Bull. Duke Univ. mar. Sta.*, Vol. 3, pp. 19–24.

Cotton, A. D., 1912. Clare Island Survey. Marine Algae. *Proc. R. Irish Acad.*, Vol. 31, Part 15, 178 pp.

Feldmann, J., 1954. Inventaire de la Flora Marine de Roscoff. *Trav. Sta. biol. Roscoff*, Suppl. 6, 152 pp.

Gibb, D. C., 1939. Some marine algal communities of Great Cumbrae. *J. Ecol.*, Vol. 27, pp. 364–82.

Greville, R. K., 1830. *Algae Britannicae.* Edinburgh.

Harvey, H. W., 1955. *The Chemistry and Fertility of Sea Waters.* Cambridge University Press. 224 pp.

Hudson, G., 1762. *Flora Anglica.* London.

Jones, W. E., 1957. A note on the genus *Cordylecladia* J. Agardh. (Algae: Rhodophyceae) *Ann. Mag. nat. Hist.*, Ser. 12, Vol. 10, pp. 70–1.

—— 1959. Experiments on some effects of certain environmental factors on *Gracilaria verrucosa* (Hudson) Papenfuss. *J. mar. biol. Ass. U.K.*, Vol. 38, pp 153–67.

May, V., 1948. The algal genus *Gracilaria* in Australia. *Bull. Coun. sci. industr. Res. Aust.*, No. 235, 64 pp.

Rosenvinge, L. K., 1931. Marine Algae of Denmark. I. Rhodophyceae. *K. danske vidensk. Selsk. Skr.* Bd. 7, 627 pp.

Spencer, C. P., 1954. Studies on the culture of a marine diatom. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 265–90.

J. mar. biol. Ass. U.K. (1958) 38, 57–63
Printed in Great Britain

THE OCCURRENCE OF *MICROCHARON* IN THE PLYMOUTH OFFSHORE BOTTOM FAUNA, WITH DESCRIPTION OF A NEW SPECIES

By G. M. Spooner
The Plymouth Laboratory

(With Text-figures 1–2)

In September 1958, Prof. J. E. Harris, F.R.S., treated freshly collected Eddystone shell gravel with a magnesium sulphate solution and so produced a rich sample of the microfauna. This has never been adequately investigated, though the macrofauna is well known. The animals collected were mostly small Malacostraca, in which Amphipoda predominated, and Prof. Harris kindly passed on to me the whole sample to examine. I have found in it thirty-four species of amphipods, isopods, tanaids and cumaceans of which no fewer than fifteen are additions to the Plymouth fauna list (see Marine Biological Association, 1957). Some indeed are new to British waters, three or four being evidently undescribed species. One of the most striking of these is described here. The collection as a whole will be reported on later.

While the animals were mainly still living, Prof. Harris mentioned having noticed a small narrow pale form of unfamiliar appearance. Occasionally one came into his view revealing possession of fairly long antennae balanced, at the posterior end, by a pair of backwardly directed processes, dilated and more or less parallel-sided. The possible identity remained a puzzle until the preserved sample had been sorted. About twenty-five specimens were eventually picked out. Their body length ranged between 1·0 and 2·8 mm., a size-range that included (above 1·8 mm) mature adults of both sexes. The structure of the pleon and its appendages showed them to be asellote isopods related to *Janira*. But they lacked eyes and pigment and (as shown by the few examples intact in this respect) possessed a most unusual development of the uropod, of which the peduncle was elongate and swollen. From these features they were readily identifiable as belonging to the blind subterranean genus *Microcharon*, as has been confirmed in detail.

THE GENUS *MICROCHARON*

In recent years various small aberrant crustaceans have been discovered living in subterranean ground water and interstitially in wet sand or gravel. Some of these (bathynellids, mystacocarids) belong to whole groups confined to this phreatic[1] realm, others (*Stenasellus*) are representative of normal freshwater families, or even, like the anthurid *Microcerberus*, of otherwise marine families. *Microcharon* Karaman, 1934, is one of a small group of genera which have such clear affinity to one or other of the marine Janiridae that they may well be included in that family (see Bocquet & Lévi, 1955), though for a time a separate family Microparesellidae was erected for them. The species included in these genera are all small and blind and most of those known to date live in terrestrial underground freshwater habitats of one sort or another.

Microcharon itself was first discovered in the Skopolje district of Macedonia, where four species (*M. stygius*, *M. latus*, *M. profundalis*, *M. major*) are known to date (Karaman, 1933, 1934, 1940, 1954). They occur well up into the mountain areas and subspeciation is already recognizable even within this territory. *M. stygius* has a subspecies *hellenae* in Greece. Another species, *M. acherontis*, is known from ground-water west of the Carpathians (Chappuis, 1944), and another unnamed from Herzegovina (Karaman, 1953). Chappuis & Delamare Deboutteville (1953) have reviewed the genus, adding *M. sisyphus* from ground-water in Corsica, and, of even greater interest, introducing two further species from coastal sand, showing that the genus extends into marine habitats. *M. marinus* was found in three localities on the southern coast of France and on the Italian coast south of Naples. *M. teissieri* (Lévi, 1950, as *Duslenia*) occurred in coarse marine sand dredged just below low-water mark off Roscoff. However, as Bocquet & Lévi (1955) have pointed out, the occurrence in bottom deposits near the coast is not conclusive evidence that the species is truly marine, since its presence may depend on seepages of terrestrial ground water bringing with it its native fauna.

It is therefore of some interest to record a third species from the sea, living in a bottom deposit sufficiently far from the shore and occurring in such a geological setting that contamination with seeping ground-water from the land seems impossible.

Microcharon harrisi sp.nov.

This species is close to *M. teissieri* (Lévi) from coarse sand near Roscoff, as shown by the detailed structure of the mouthparts and other appendages. In particular, the form of male pleopod 1 is identical and pleopod 3 bears three plumed setae. The differences are seen in the larger size of *harrisi*, its relatively more elongate mesosome segments, its very distinctly longer antenna, and its somewhat more elongate pereiopods and uropod. *M. teissieri* has been well described and figured by Lévi (1950) and Chappuis & Delamere Deboutteville (1953). Various details shared in common between the two species are not necessarily repeated below.

---

[1] A convenient (French phréatique) name for underground waters of all types, ranging from cave pools to interstices between coarse soil particles.

The body length of adults (i.e. length excluding antennules, antennae and uropods) is 1·8–2·8 mm, hence longer than other species except possibly *M. major* Karaman, and between 1·5 and 2·0 times the length of *M. teissieri*. The corresponding mean body width is 0·18–0·28 mm, i.e. one-tenth of the length. (This assumes that no artificial pressure is applied, as by a coverslip on a mounted specimen. Also the loose intercalary connexions of the segments allow about a ± 15 % expansion or contraction from the mean length.) Middle body segment about 85 % the width of the head and pleotelson. See Fig. 1A.

Head subrectangular, somewhat narrowed posteriorly, with mean dorsal length 1·1 to 1·2 times the central width; a short rounded prominence anteriorly between the antennae. No vestiges of eyes.

Pereion segments typically of proportions shown in Fig. 1. Segment 1 about half length of head; segments 4 and 5 may be a little shorter than wide, the rest at least as long as wide, longer in the larger animals. (In *M. teissieri* all segments are described as shorter than wide.) There is a graded narrowing of mean width from both ends towards the middle, but only to an extent of about 10 %.

The separate first pleon segment is 0·4 to 0·6 the length of the last pereion segment. The pleotelson is usually about the same length as the head, but is distinctly longer in the larger individuals.

The antennula has five segments. This, with the mandible, maxilla and maxillipede of a 2·10 mm male are shown in Fig. 2. The antenna drawn is that of a 2·05 mm female; this limb is normally 3·2 to 3·4 times the length of the head, hence about twice as long (relatively) as described in *M. teissieri*, and furnishing the most obvious difference from that species (it is longer than the head + the first three mesosome segments). The squama on the third segment bears one or two long setae.

The pereiopods (Fig. 2E) are more elongate than in *M. teissieri*, though the difference is small in the younger adults.

The male pleopods (Fig. 1B, Fig. 2F, G, H) are as described for *M. teissieri* except that in pleopod 2 the distal region is shorter and less acute apically, conforming more nearly to the illustration of this limb in *M. marinus* Chappuis & D. Deboutteville. Pleopod 3 (Figs. 1C, 2H) of both sexes bears three stout plumed setae, as does *M. teissieri*, but not any other known species.

The enlarged peduncle (sympode) of the uropod (Fig. 1A, D) seems typically to be close to 1·4 times as long as the pleotelson (1·5 times in one male). This is relatively a little larger than in *M. teissieri*, *M. marinus* and *M. major*, and so is larger than in any of the species yet described. The endopodite is 0·30 to 0·33 the length of the peduncle, and the laterally directed exopodite is about half the length of the endopodite.

Females bear two eggs or young in the mesosomic ventral brood-pouch, as described in other species, and carry them up to the length of at least 0·85 mm (i.e. about half the body length of the mother).

Type locality: North of Eddystone Rock, in shell gravel, below depth of 23–28 fm. The site is 9 miles from the nearest point to the mainland.

Type specimens will be deposited in the British Museum.

Over seventy examples have now been examined. Only eight, however, of these retain one or both uropods, and only twenty-one possess one or other of the antennae. (The most complete specimen, the adult female figured, carried both uropods and one antenna.) The antennae easily break off at the end of the 4th peduncle segment. Other limbs are not readily shed. This liability to lose the uropod and most of the antenna has been noted in other species, though *M. teissieri* is said to be much less liable to damage.

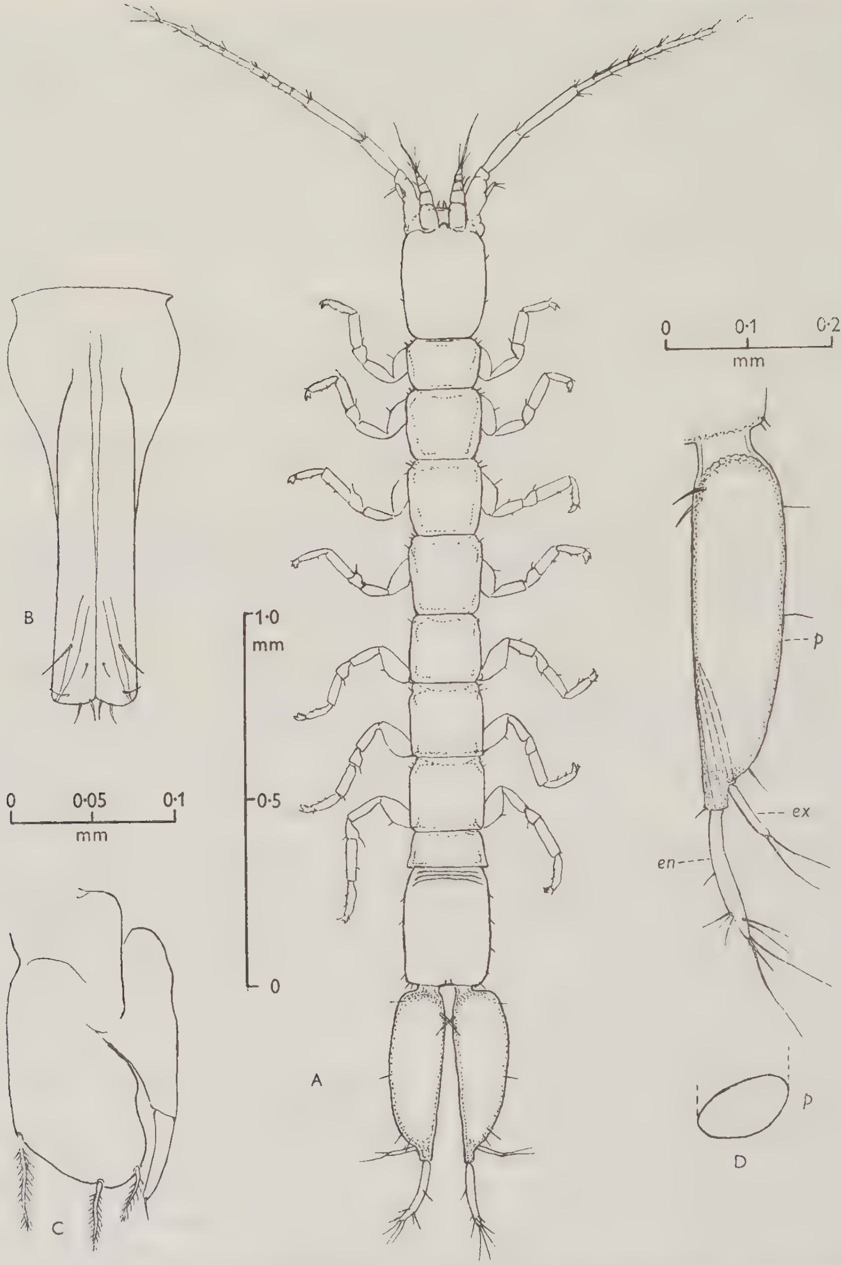

0
0·1
0·2
mm
B
1·0
mm
0·5
0
p
0
0·05
0·1
mm
ex
en
A
p
D
C

Fig. 1. *Microcharon harrisi* sp.nov. A, adult female of 2·05 mm body length. B, pleopod 1 of male of 2·10 mm. C, pleopod 3 of a female of *ca.* 2·3 mm. D, uropod of male of 2·04 mm, with section through middle given below, to show thickness: *p*, peduncle; *ex*, exopod; *en*, endopod.

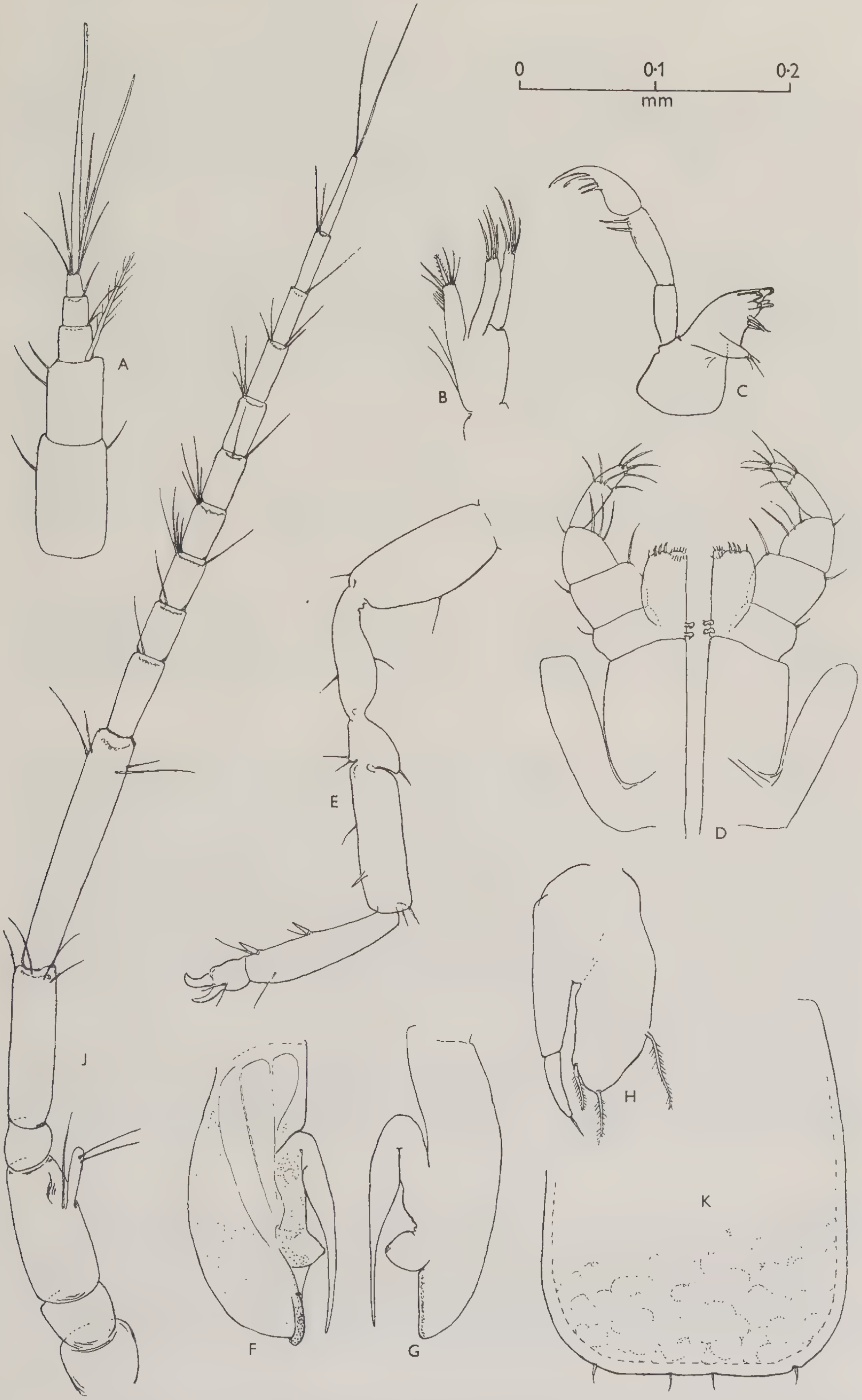


Fig. 2. *Microcharon harrisi* sp.nov. Limbs of male of 2·10 mm: (A) antennula; (B) maxilla; (C) mandible; (D) maxillipede; (E) pereiopod 7; (F, G) pleopod 2, right and left from somewhat different angles; (H) pleopod 3. J, antenna of female of 2·05 mm. K, female operculum (fused pleopod 1 pair).

FAUNISTIC RELATIONSHIPS

In the accompanying fauna an even more surprising find has been a species of bogidiellid amphipod—a family so far known from 4 or 5 small fragile species living in terrestrial ground-water and shore sand in southern Europe and South America. (The Plymouth species is being described as type of a new genus related to the existing *Bogidiella*.)

The occurrence of these two typical phreatic interstitial forms in an offshore marine deposit raises an issue. It suggests that the underground fauna contains a 'cosmopolitan' element that is not simply confined to the ground-water of the land masses, but extends laterally under the sea in bottom strata and deposits, in the sense that its geographic dispersal is primarily below the surface of the ground or sea-bed. A fuller discussion of this topic is reserved for later.

If such a picture should be correct, there would be no difficulty in explaining why various animals concerned are related to otherwise marine groups and evidently derived from marine ancestors. *Microcharon*, if not indeed the whole subfamily Microjaniridae (in which it is placed), provides a typical example.

It must be emphasized that there are big gaps in our knowledge of the smaller fauna of marine deposits, especially that living below the top few centimetres. This would clearly repay further study if only for testing the above hypothesis.

SUMMARY

The Eddystone shell gravel fauna includes a species of *Microcharon*, a genus of small blind janirid isopods, first known from terrestrial ground-water in southern Europe, and more recently discovered in coastal marine sand.

The species concerned is related to *M. teissieri* (Lévi) from Roscoff, but its larger size, longer antenna, and more elongate body proportions show it to be distinct. It is named *M. harrisi* after Prof. J. E. Harris, who concentrated the gravel microfauna and noticed the animal alive before the sample was preserved.

The genus is now confirmed as genuinely marine, and may with good reason be regarded as of marine origin.

The occurrence of *Microcharon* and a bogidiellid amphipod in an offshore deposit suggests that the domain of the phreatic fauna—i.e. that inhabiting the ground-water below the land surface—actually extends below the sea bed.

REFERENCES

Bocquet, C. & Lévi, C., 1955. *Microjaera anisopoda* nov.gen., n.sp., isopode psammique de la sous-famille nouvelle des Microjanirinae. *Arch. Zool. exp. gén.*, T. 92, N. & R. No. 9, pp. 116–34.

Chappuis, P.-A., 1944. Die Grundwasserfauna der Körös und des Szamos. *Math. term. Közl. Budapest*, 1944, pp. 5–44. (Not seen.)

Chappuis, P.-A. & Delamere Deboutteville, C., 1953. Recherches sur les Crustacés souterrains. VII. Les Isopodes psammiques de la Méditerranée. *Arch. Zool. exp. gén.*, T. 91, Fasc. 1, pp. 103–38.

Karaman, S., 1933. Neue Isopoden aus unterirdischen Gewässern Jugoslaviens. *Zool. Anz.*, Bd. 102, pp. 16–22.

—— 1934. Beiträge zur Kenntnis der Isopodenfamilie Microparasellidae. *Mitt. Höhlen u. Karstforsch.*, 1934, pp. 42–4. (Not seen.)

Karaman, S. 1940. Die unterirdischen Isopoden Südserbiens. *Bull. Soc. Sci. Skoplje*, T. 22. (Not seen.)

—— 1953. Über subterrane Amphipoden und Isopoden des Karstes von Dubrovnik und seines Hinterlandes. *Acta Mus. macedon. Sci. Nat.*, T. 1, No. 7, pp. 137–67.

—— 1954. Weitere Beiträge zur Kenntnis der microparaselliden Mazedoniens, das genus *Microcharon* Karaman. *Fragmenta balcanica*, T. 1, No. 12, pp. 108–14.

Lévi, C., 1950. *Duslenia teissieri*, nov.gen., n.sp., nouveau Parasellidé des côtes de France. *Arch. Zool. exp. gén.*, T. 87, N. & R., No. 5, pp. 42–7.

Marine Biological Association of U.K., 1957. *Plymouth Marine Fauna.* Third edition.

J. mar. biol. Ass. U.K. (1959) 38, 65–80
Printed in Great Britain

THE REPRODUCTION AND LARVAL DEVELOPMENT OF *NEREIS FUCATA* (SAVIGNY)

By J. B. Gilpin-Brown
From the Plymouth Laboratory

(Text-figures 1–7)

The wide variety of reproductive patterns and behaviour in the many species of Nereidae already studied clearly justifies further research. But the life history of *Nereis fucata* (Savigny) is not only of interest from the comparative point of view. Its commensal habit (it occurs within shells occupied by hermit crabs) immediately gives it a special importance. This alone warrants a detailed study, particularly as no commensal polychaete has yet been reared through to metamorphosis and settlement on its host (Davenport, 1955; Davenport & Hickok, 1957). The numerous interesting problems which arise, and the experimental methods needed to study them, are, however, beyond the range of a paper on nereid development. It is therefore proposed to confine the present account to the reproduction and development up to the time when the larvae settle on the bottom. The complete life cycle, the mechanism of host-adoption, and related topics, will be reported in later papers.

MATERIAL AND METHODS

Many of the shells occupied by hermit crabs in the Plymouth area are also occupied by *Nereis fucata*. It is a large and easily recognized nereid, but a few specimens have been checked against the description given by Fauvel (1923) for this species. Ripe heteronereids were collected from Plymouth trawling grounds during 1955 and 1956. The area extended as far as the Looe Grounds, the Eddystone Inner Channel Grounds, and Bigbury Bay. All were from otter-trawl hauls except a few from the Rame Mud area where an Agassiz trawl was used.

Artificial fertilizations were made in the usual way. Clean oocytes were obtained by partially stripping a female. They were then thoroughly washed in three changes of water. Sperm was produced when a few oocytes were added to a dish containing a ripe male. The time when the oocytes and sperm were mixed has been taken as the time of fertilization. After fertilization the eggs were washed, either by allowing them to sink through a long column of water, or else by three changes of filtered 'outside' sea water. Each culture was divided amongst as many bowls as possible. A few of these were then kept at laboratory temperature for close study, while the remainder were placed in cold rooms at 11° and 12–13° C. The after-care of the cultures was substantially that described by Just (1922). In the young stages food consisted of *Phaeodactylum* and *Isochrysis* cultures.

At frequent intervals 35 mm photographs were taken of each culture in order to obtain a continuous record of size and the grosser morphological changes (number of setigers, etc.). At less frequent intervals detailed drawings were made with a camera lucida. The very fine details were usually added by eye afterwards. Nicotine was the most useful narcotic for the young ciliated stages, but magnesium chloride was needed as soon as much muscular movement began. Details of the parapodia were obtained from larvae fixed in Bouin and then cut up under a dissecting microscope. Each parapodium was then mounted directly in polyvinyl pyrrolidine and drawn with a camera lucida.

REPRODUCTION

At maturity *Nereis fucata* undergoes a 'complete metamorphosis' (Herpin, 1926) to form a typical heteronereid with an anterior unmodified region and a posterior natatory region. It has been fully described and figured by Fage (1904), and Charrier (1921) has given a detailed account of the internal modifications which take place.

Mature worms from shells housing *Eupagurus bernhardus* were obtained in gradually increasing numbers from March to June in 1956. The numbers then rapidly declined, and in both 1955 and 1956 no mature worms were collected after the middle of June, while two females with degenerating oocytes were obtained on 30 June 1955. Breeding therefore occurs sometime during the period from April to the beginning of June. More detailed information has been difficult to find. There is some evidence to suggest that the main breeding season is between the middle of May and the middle of June, since the percentage of heteronereids dropped from 4 to 0·1 % during this period. However there is no record of nereid eggs or larvae in the plankton at this time, although an examination was made of thirty standard hauls taken between 18 April and 28 June from the Looe, Looe-Eddystone and Rame-Eddystone grounds. This suggests that the eggs and larvae are not in the upper layers. On four occasions from 16 to 30 May the early part of the night was spent over the grounds with a submerged light, but no heteronereids were seen. Since this species occurs in 30 fathoms or more it is possible that the swarming and development of the eggs takes place near the bottom. It is presumed that *N. fucata* normally leaves the hermit crab and swarms, as do other nereids, since no spent worms have been collected and because ripe worms swarm readily in the laboratory. Their behaviour is exactly similar to the swarming nereids described by Herpin (1926).

These mature worms are conspicuously coloured as a result of the usual morphological changes associated with epitoky. The body wall loses its pigment until the white sperm and coloured oocytes are clearly visible, and the vascularization of the parapodia becomes so increased that a red colour predominates. In *N. fucata* the creamy-white pre-natatory region of the male is divided down the middle by the bright red dorsal blood vessel. In the natatory region this order is reversed since the blood vessel disappears leaving a broad white band in the mid-line, bordered on either side by the rose-red parapodia. The female coloration, on the other hand, is much more variable because it is dependent on the colour of the oocytes. In *N. fucata* the females are either lilac, turquoise, or blue. As in the male the parapodia add a pink border down either side of the natatory region.

The sexes are therefore very easy to distinguish and 47% of the heteronereids collected during this period were males. This probably indicates a 1:1 sex ratio. This seems to be the normal ratio for epitokous species since Reish (1954) got a 1:1 ratio in his cultures of *N. grubei*, and Takahasi (1933) reported a 5:4 ratio in *Perinereis nuntia brevicirris*. In some atokous species (e.g. *Nereis diversicolor*) the ratio may be less (Dales, 1950; Bogucki, 1953).

Ripe males were readily stimulated by the presence of a few oocytes, shedding sperm within 30–40 sec. This could be repeated after rinsing the male in clean water, as described by Herpin for *N. irrorata* and *Perinereis marionii*. When both sexes were present ovulation usually occurred after the sperm was shed, but this was less consistent. *Nereis fucata* seems to resemble *N. succinea* closely in this respect (Lillie & Just, 1913, as *N. limbata*). In an aquarium sperm streams from the posterior end, apparently from the anus, but possibly from anal papillae (Defretin, 1949). The oocytes pour from the undersides of the last few segments. Many species shed their eggs through rents in the body wall (e.g. *Platynereis megalops*, Just, 1914; *Nereis grubei*, Reish, 1954).

EMBRYONIC DEVELOPMENT

The mature oocytes rapidly acquire a smooth regular outline when released from the female, and assume a diameter which varies between 200 and 250 μ, the range for any one female being about 20 μ. The average oocyte diameter for this Plymouth population was 225 μ. This is a little on the large side for most epitokous nereids, but compares favourably with the epitokous form of *Perinereis cultrifera*, a worm of approximately similar size (Herpin, 1926). The lilac, blue, or turquoise colour is obtained from numerous small oil droplets which are visible within the oocyte. The range of colour is presumably due to varying proportions of the turquoise and purple pigments distinguished by Green & Dales (1958).

The first indication of a successful fertilization is a separation and a piling up of the eggs on the bottom of the culture dish. This is due to the rapid formation of a gelatinous envelope round each egg. At 15° C it first appears about 15–30 min after fertilization. The pile of eggs then begins to resemble diminutive frog-spawn, and this resemblance increases during the next 2½ h until each egg is almost 1·5 mm in diameter. A thick non-adhesive envelope is a characteristic feature of those nereids with pelagic larvae. It is not adhesive in *Nereis fucata*. The elevation of the fertilization membrane is quite normal and first appears 1–2 h after fertilization at 15° C.

The embryology of the nereids has been described and figured by E. B. Wilson (1892). More recent workers have shown that all the species closely follow Wilson's description despite differing amounts of yolk in the different species: (compare, for instance, the pelagic eggs of *N. japonica* (150 μ, Izuka, 1908) and the incubated eggs of *N. caudata* (420–520 μ, Reish, 1957)).

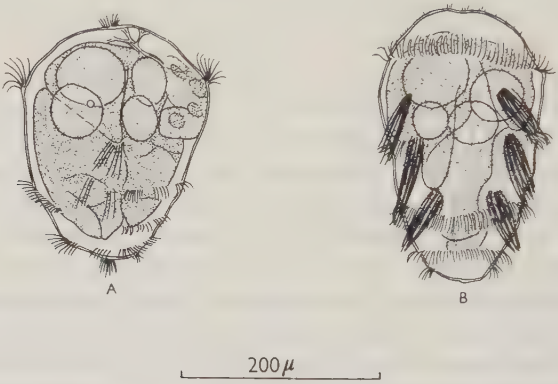


Fig. 1. A: trochophore; lateral view of the right side. Only the margins of the prototroch are shown. B: late trochophore; dorsal view.

In *N. fucata* the first cleavage occurs 4½–5 h after fertilization at room temperature, and is closely followed by the second and third (6–7 h). Epibolic gastrulation proceeds in the normal way, and after about 12 h can be easily seen, since the small micromeres form a colourless cap of cells on top of the large coloured macromeres. By the end of the first day gastrulation is complete and sufficient cells have been formed to give a smooth regular outline to the embryo. With the further multiplication of cells the embryos continue to gain in compactness and regularity, but they remain more or less spherical with the fertilization membrane still standing out from the surface in places. Spasmodic rotation usually begins after about 33 h, although cilia cannot be seen. The prototroch first becomes visible some 3 h later, when the embryos are actively rotating. It is almost complete on the second day, and its cilia are large and conspicuous by the end of 3 days when the embryos have developed into monotrochophores. The typical shape has been achieved by an elongation of the body and some slight local swelling equatorially at the base of the prototroch.

During the next 24 h the larvae develop into complex and highly motile trochophores (Fig. 1A). The increase in motility is most marked and is derived from additional ciliary bands, especially the ventro-lateral paratrochs. In some larvae a band of pigment is formed at the base of the prototroch. Internally, continued differentiation has produced longitudinal muscles, shown by an occasional muscle twitch, and six pairs of setal bundles are clearly visible (Fig. 1B). It seems very probable that in nature hatching normally takes place at this stage, since it did so in all cultures kept between 11° and 13° C. In these cultures, hatching is only prevented by the presence of the gelatinous envelope. This disappears 4–6 days after fertilization irrespective of temperature so that larvae reared at 11° C hatch at an earlier stage (trochophores) than those reared at 15° C (3-setigers). As hatching is therefore not directly dependent on temperature or development, a comparison with other nereids is difficult. In general terms, however, *N. fucata* corresponds with other epitokous forms in having a shorter embryonic period than the atokous forms.

LARVAL DEVELOPMENT

Although swimming persists, the next stage (Fig. 2A) is characterized by the presence of long setae. These are highly mobile and each setiger is composed of noto- and neuropodial bundles. In *N. fucata* (at 15° C) their emergence occurs about 5 days after fertilization. This is rather later than in many species (e.g. 30 h in *N. irrorata*, see Herpin, 1926), but resembles *N. pelagica* (4½–5 days), which was also reared at Plymouth (D. P. Wilson, 1932). In *N. fucata* all three setigers are formed at about the same time, although the second may appear first, followed closely by the first and third. This order, however, is apparently determined only by the length of the setae in each setiger, which is in contrast to some species where there is a definite time lag before the formation of the third setiger (e.g. 20 h in *N. succinea* and *Platynereis megalops*, see E. B. Wilson, 1892).

At first these larvae are simply setigerous larvae (Fig. 2A), but during the next few days a gradual change takes place to form young 3-setiger worms (7 or 8 days at 15° C). Externally the features (Fig. 2B, C) which are most responsible for this change are: an increase in length, emphasized by the development of a pair of very small anal cirri; and, perhaps most characteristic, the formation of distinct parapodia, beginning as small lobes between the noto- and neuropodial bundles of setae. The rudiments of the cephalic appendages, each bearing sensory hairs (E. B. Wilson, 1892), then become prominent at about daily intervals. The anterior tentacular cirri are the first to appear, followed by the antennae, and, towards the end of this stage, a pair of small pimples on either side of the mouth which represent the future palps (they lie immediately beneath the prototroch in Fig. 2C). Two irregularly shaped patches of pigment are also often formed ventro-laterally on either side of the head. These, however, are very variable and some larvae may not have them. Internally, blocks of tissue appear representing the proboscis, the large yolk-filled mid-gut, and the hind-gut.

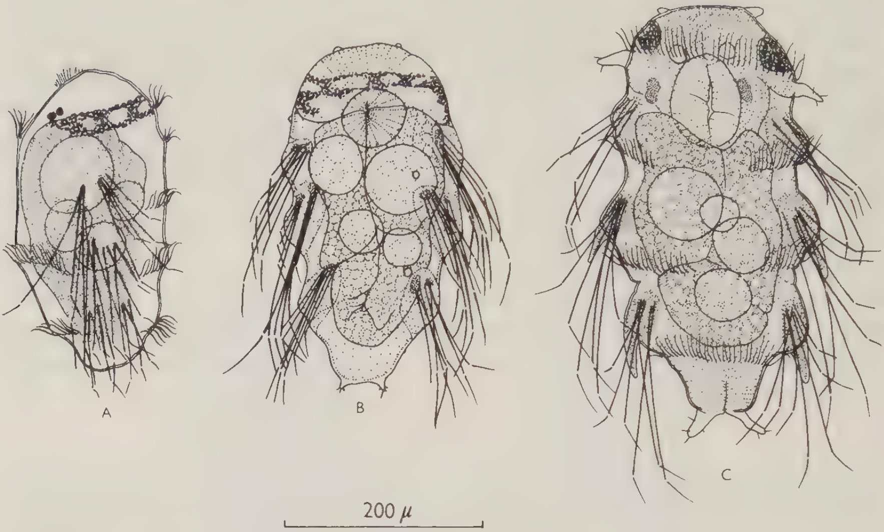


Fig. 2. A: setigerous larva; lateral view of right side. Only the margins of the prototroch are shown. B: early 3-setiger larva; ventral view. No cilia were seen in this specimen. C: 3-setiger larva; ventral view.

The growth rate now becomes a little more variable, and the cultures begin to contain larvae at different stages. At 15° C the fourth setiger is formed some time between 6 and 11 days after fertilization, while at 11° C it appears at 15–23 days. At first only a few setae project, but these rapidly increase in length and number (Fig. 3 A). The cephalic appendages, the anal cirri, and all the parapodial lobes continue to grow in size. As in *Nereis pelagica* (see D. P. Wilson, 1932) and *N. costae* (see Durchon, 1956), the minute jaws become visible at this stage, and the gut, especially the proboscis and hind-gut, become more differentiated. In the 5-setiger stage (Fig. 3 B), which occurs at about 2 weeks at 15° C and at about 3 weeks at 11° C, this process of elaboration continues. The setae are now very long and all the parapodia bear distinct noto- and neuropodial lobes with ventral cirri on the second to the fifth. In addition, the jaws and the proboscis can be everted and the gut is sufficiently formed for the larvae to swallow food, although yolk reserves are still present in the mid-gut. The cilia remain very active, but their disappearance has begun with the division of the prototroch into two lateral bands.

The appearance of the sixth setiger, which occurs at about 18 days, marks the beginning of the end of the larval period. By the time another setiger has been formed (at 26 days) the larvae have become juveniles and have commenced a crawling life upon the bottom (Fig. 4). Associated with this change

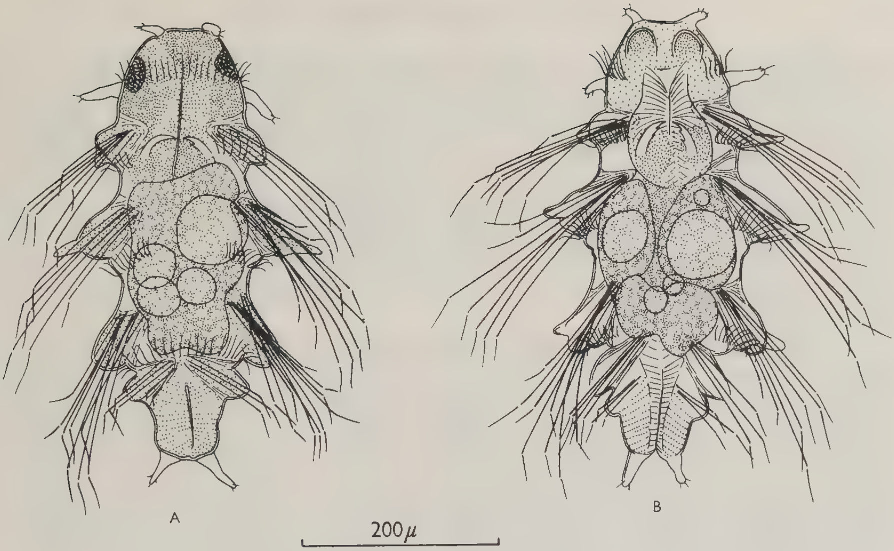


Fig. 3. A: 4-setiger larva; ventral view. B: 5-setiger larva; ventral view.

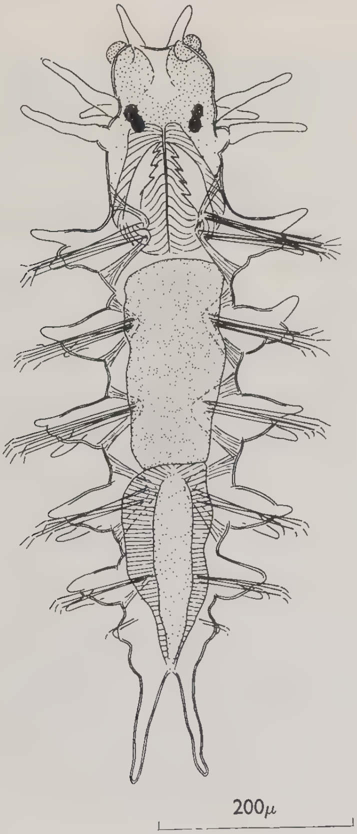


Fig. 4. 6-setiger (7-segmented) larva; dorsal view.

is the loss of the cilia and of the long setae in the first three setigers. In addition the parapodium of the first setiger rapidly develops into the posterior tentacular cirrus (see below). On the head the ventral anterior tentacular cirri begin to appear, and the palps now project forward in the adult position. Thus all the cephalic appendages are now present except for the ventral posterior tentacular cirri which appear some time later. From now on the larvae make small tubes on the bottom of the culture dishes and are dependent upon external sources of food; if they are under-fed the length actually decreases after this stage.

In *N. fucata*, therefore, settlement, feeding, and the cephalization of the first setiger occur at approximately the same stage of development. In many nereids this is not so, for crawling is often immediate when the eggs are incubated (*e.g. N. costae*, see Durchon, 1956) and, since such eggs are frequently more heavily yolked, feeding is correspondingly retarded (e.g. to the 20-setiger stage in *N. caudata*, see Herpin, 1923). On the other hand, cephalization normally occurs in 6-setiger larvae, although *N. lightii* (4-setiger; Smith, 1950) and *N. diversicolor* (9-setiger; Dales, 1950) are exceptions.

CILIATION

The ciliation of the various larval stages of *N. fucata* is set out diagrammatically in Fig. 5. In this species all the components of the basic nereid pattern are present at one stage or another; an akrotroch, a prototroch, three paratrochs, and a telotroch. In addition, occasional tufts of cilia may occur in other positions in certain larvae (e.g. the ventral tuft immediately behind the prototroch in Fig. 2A).

An akrotroch is only present in the trochophore, in contrast to *N. pelagica* where it remains until the 4-setiger stage (D. P. Wilson, 1932). An incomplete prototroch is almost certainly present when the embryos are rotating, but it is probably only completed in the monotrochophore. It then persists as a complete girdle until gaps appear, at first dorsally and then ventrally, in the 5-setiger stage (Fig. 5). In common with all the ciliary bands it disappears completely in the 6-setiger larvae. Three paratrochs are a regular feature in nereids, being present even in the non-swimming larvae of *N. costae* (see Durchon, 1956) and in the viviparous *N. lightii* (see Smith, 1950). In *N. fucata* they begin in the trochophore as short rows of cilia (Fig. 1A) which then join up to form (in the second and third) two complete rings. There is then a fairly steady regression, the posterior ones being a little more persistent (Fig. 5). A telotroch is not usually present in nereids, though it occurs in *N. diversicolor* (see Dales, 1950). In *N. fucata* this does not last long enough to show whether it is a true telotroch or a fourth paratroch.

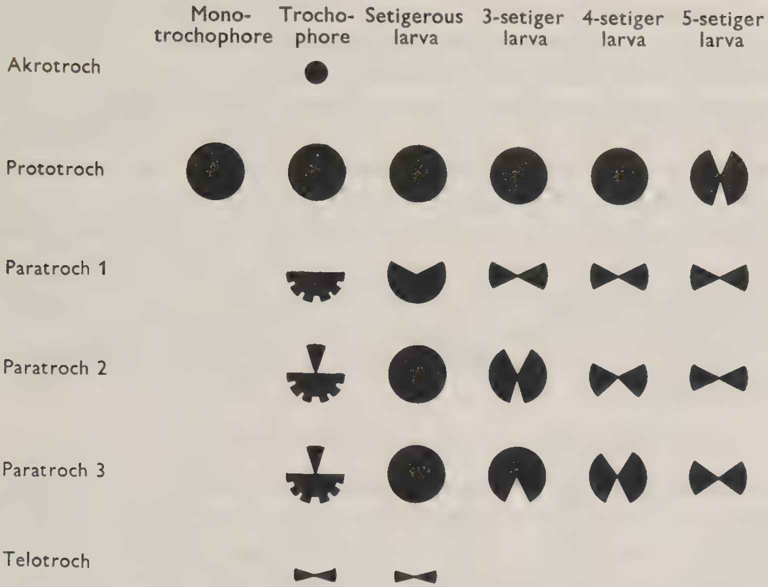

Mono-trochophore
Trocho-phore
Setigerous larva
3-setiger larva
4-setiger larva
5-setiger larva
Akrotroch
Prototroch
Paratroch 1
Paratroch 2
Paratroch 3
Telotroch

Fig. 5. Diagram of the larval ciliation. The black segments show the extent of each ciliary band at the various larval stages.

COLOUR

Pigment first appears in small patches around the prototroch (Fig. 2A, B). In the 3-setiger stage it has disappeared, but the majority of the larvae have two reddish patches ventro-laterally on either side of the head (Figs. 2C, 3A), which begin to diminish at the 5-setiger stage. The larvae are practically colourless at seven setigers. Irregularly shaped patches of pigment on the head are fairly common in this family. E. B. Wilson (1892) reported them first in the 3-setiger stage of *N. succinea*, where they were derived from the equatorial band of the trochophore. They are also present in the epitokous form of *Perinereis cultrifera* (see Herpin, 1926) and in some 3-setiger larvae of *Nereis pelagica* (see D. P. Wilson, 1932). They are not confined to the epitokous forms since they have also been reported in the 4-setiger larvae of both *N. costae* (see Durchon, 1956) and, occasionally, *N. diversicolor* (see Dales, 1950).

PARAPODIA AND SETAE

The early stages in the formation of the parapodia are very similar to those described and figured by Finke (1936) for *N. diversicolor*. A very small lobe between the noto- and neuropodial setae first appears in the setigerous trochophore. During the succeeding stages this increases in length and forms the notopodial lobe in the first two adult parapodia. In the 3-setiger stage the neuropodial setae are borne on a small prominence which later divides to give the ligules and ventral lobe. Ventral cirri are present in the 6-setiger larva, when the aciculae are also clearly visible.

The larval setae follow much the same succession as those of *N. pelagica* (see D. P. Wilson, 1932), with appendices varying between 20 and 60 μ in length, so that there is no real distinction between falcigers and spinigers. The 3-setiger larva has 6–7 homogomph setae in both rami of the first parapodium. In the 5-setiger stage these are reduced to about 4 or 5, while they are lost altogether when the parapodium metamorphoses in the 6-setiger larva. The second and third setiger bear, in addition to the homogomph setae, a simple capillary in both noto- and neuropodia. In the transition stage all the notopodial setae are lost and heterogomph falcigers begin to appear in the neuropodium.

DEVELOPMENT OF THE POSTERIOR TENTACULAR CIRRI

The development of the posterior tentacular cirri from the first larval parapodium is set out in Fig. 6. This clearly shows the elongation of the notopodial lobe to form, in the 6-setiger larva, the dorsal tentacular cirrus. Since Langerhans' (1879) description of the development of *Platynereis dumerilii*, it is usually assumed that these cirri are derived from the dorsal and ventral cirri of the first parapodium. In *Nereis fucata*, however, this is not strictly true, for it can be seen that the dorsal tentacular cirrus develops from a lobe which is inferior to the notopodial setae at a time when the parapodium has no dorsal cirrus. *N. fucata* therefore agrees with *N. pelagica* in which the dorsal tentacular cirrus is derived from the middle lobe of the parapodium (D. P. Wilson, 1932). It probably also agrees with *N. diversicolor* (see Finke, 1936), and may further agree with *N. vexillosa* (see Johnson, 1943), *N. grubei* (see Reish, 1954), *N. succinea* (see Banse, 1954), and *N. lightii* (see Smith, 1950), since the derivation of this cirrus is not considered in detail. Many other species, however, resemble *Platynereis dumerilii* (see Hempelmann, 1911), where this cirrus is formed from the dorsal cirrus of the first parapodium (e.g. *Nereis caudata*, see Herpin, 1926; *N. costae*, see Durchon, 1956; *Perinereis marionii*, see Herpin, 1926; and *Micronereis variegata*, see Rullier, 1954). E. B. Wilson (1892), on the other hand, records that in *Platynereis megalops* and *Nereis succinea* the first parapodium never carries parapodial cirri.

GROWTH OF THE LARVAE

Although weight would be the best indication of growth and growth rates, its measurement is difficult and it has not been used for larval nereids. Length is more practical and provides a reasonable indication. It has been widely used, and the figures given by Bogucki (1953) for *N. diversicolor* give the usual sigmoid growth curve. In the same species Dales (1950) has shown that in the early stages (0–9 weeks) length increases at a constant rate of 0·04277 % per day. It will be seen from the graph (Fig. 7) that in the young stages of *N. fucata* the increase in length occurs at two speeds; an initial high rate during the first week, followed by a much slower one (Table 1). The length measured excludes appendages.

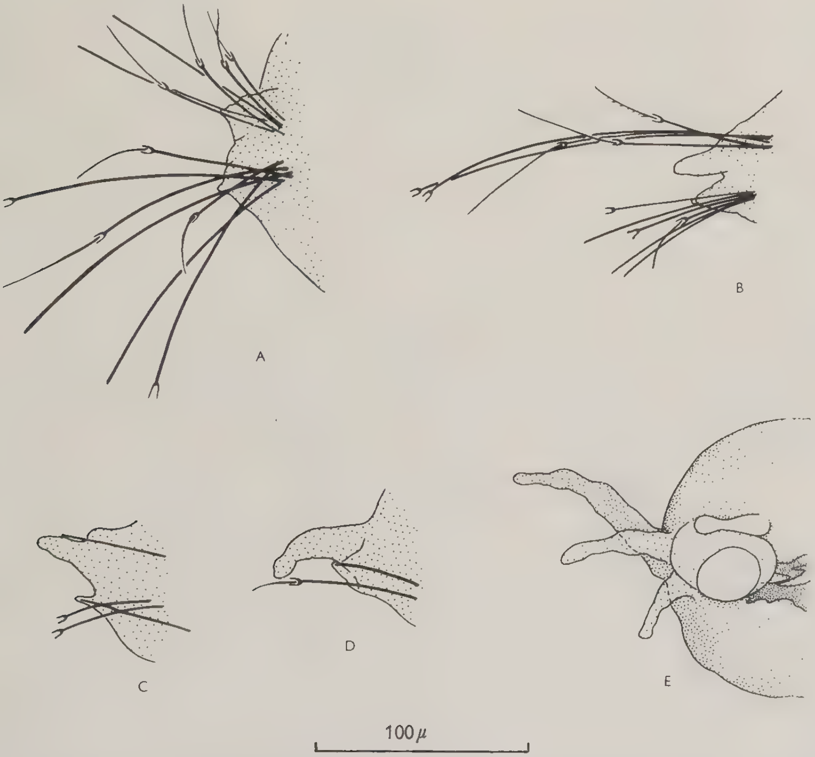


Fig. 6. Stages in the cephalization of the first parapodium. A: 3-setiger larva. B: 4½-setiger larva. C: 6-setiger larva, setae falling out. D: 6-setiger larva; elongation of the notopodial lobe. E: 6-setiger larva; frontal view after cephalization is complete.

The formation of segments is a natural and simple method of following development and has been recorded in most species. The figures for *N. fucata* are also given in the table and are in broad agreement with other species, though a close comparison is difficult. Segment-formation cannot be a strict measure of growth since it is always positive and may cease at a relatively early stage. Thus in *N. grubei* the production of segments stops when 8–85 % of the final weight has been reached (Reish, 1954). In agreement with the high positive correlation between length and segments in the young larvae of *N. diversicolor* found by Dales (1950), their curves are a close fit, at least in the early stages. It seems likely that this similarity only holds good while the embryonic yolk reserves last. The figures provided by Hempelmann (1911) for individual 'nereidogene' larvae of *Platynereis dumerilii* (since referred to *P. massiliensis* by Hauenschild, 1951) confirm this, for they show that after this stage the rate of segment formation varies considerably.

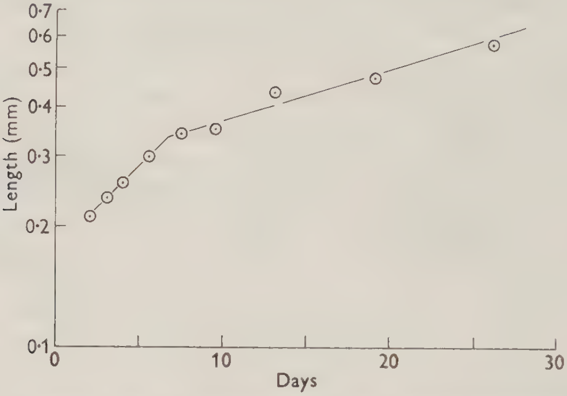


Fig. 7. Length of the larvae during the first month after fertilization.

TABLE 1. GROWTH OF LARVAE

Stage	Age (days)	Length (μ)	Growth (%/day)
Rotating embryos	2	220	0·10449
Monotrochophores	3	235	
Trochophores	4	256	
Setigerous larvae	5	301	
3-setiger	7	340	0·02719
4-setiger	9	350	
5-setiger	13	435	
6-setiger	18	473	
6-setiger (7-segmented)	26	570	

DISCUSSION

The development described in this paper refers to worms kept and reared in the laboratory. There must remain, therefore, some uncertainty about their behaviour and development under natural conditions. The rate of development is probably by far the most variable character and will presumably be mainly determined by temperature. The larvae are probably near the bottom, where the temperature will increase from about 9° C in May to about 12° C in July. It follows that the record of larvae reared at 11° C will give the best indication of their normal rate of development; but this remains a very rough approximation. This inaccuracy is further increased since, after settlement, growth will be closely linked with the availability of their natural food; and this, and its effects, are completely unknown. However, subject to these limitations the reproduction and early life history of *N. fucata* at Plymouth is probably as follows.

As no free-living population of this commensal is known (reports probably refer to the occasional single specimen, e.g. Hornell, 1891), the species may be presumed to come to maturity slowly, because only some 10% of the worms collected were mature. At maturity the development of the definitive heteronereid characters takes place while the worms are still within the shells of the host crabs. Then, probably in the month beginning in the middle of May, these brilliantly coloured worms come out of their shells to swarm in a normal nereid manner, perhaps just off the bottom. It is not known whether this is periodic or not, but some synchronization mechanism would seem necessary. The actual spawning and fertilization mechanism is again the same as in other epitokous species. Blue, lilac, or turquoise eggs are produced which, when fertilized, have the usual thick gelatinous envelope. The embryonic development is normal and highly motile larvae, with abundant cilia, are formed. These may swim around on or near the bottom, finally settling on to it when six setigers have been developed. The behaviour of the young worms in the laboratory strongly suggests that, some 4–6 weeks after fertilization, they will be living and feeding on the bottom within small tubes they have constructed. Their precise habitat, if they have one, is unknown. Young *N. fucata* have been recorded from Rame Mud, after sieving through stramin netting (Mare, 1942), but attempts to repeat this have been unsuccessful. However, McIntosh (1910) records them from *Filograna* and from stones and shells in deep water, so that they appear fairly widely distributed.

The reproduction and larval development is therefore very similar to the other members of the family. In particular, the feeding and tube-building habit is quite normal. Up to this stage, therefore, no special adaptation to commensalism can be seen. The young probably settle on the bottom first and enter hermit crab shells later. Davenport (1955) has discussed this problem of settlement in commensal polychaetes and has suggested that the young stages may first settle on the same substrate as their hosts. This would increase their chances of finding a host, and the development of a specific host reaction would then quickly lead to its adoption. *N. fucata* therefore fits the first part of this hypothesis very well, although it is not known if there is a preference for a particular substrate. This can only be determined by further experiments, but it seems possible that a high degree of selection may not be necessary for such motile and widespread hosts. The next problem is to determine the specific reaction which ensures the adoption of the host. Preliminary experiments suggest that in this respect *N. fucata* also fits Davenport's hypothesis.

It is a pleasure to record my gratitude not only to the Director and all his staff at the Plymouth Laboratory for their generous help during the course of this work, but also to the Council of the British Association for allowing me the use of their table. My thanks are due in particular to Mr P. G. Corbin for first drawing my attention to the mature heteronereids, to Dr M. W. Parke for supplying the *Isochrysis* and *Phaeodactylum* cultures, to Mr A. C. G. Best for the use of his polyvinyl pyrrolidine mountant, and to Mr G. R. Forster for so willingly taking me over the grounds at night in his yacht 'Sunset'.

SUMMARY

The reproduction and larval development of *Nereis fucata* (Savigny) is described up to the time of settlement (6- to 7-setigers).

Mature heteronereids are found at Plymouth from March to June, in a 1:1 sex ratio. In an aquarium the spawning mechanism is similar to other species.

The embryonic development is typical of an epitokous species with pelagic development. The eggs, whose mean diameter was 225 μ, are lilac, blue, or turquoise in colour. Cleavage and gastrulation are normal. Rotation begins at 36 h and monotrochophores are formed after 3 days. Hatching probably occurs 4–6 days after fertilization when a highly motile trochophore has developed.

The setae of the first three segments appear after about 5 days. The differentiation of the parapodia, and the larval succession of setae, are essentially the same as in other species.

Settlement, the cephalization of the first parapodium, the loss of the cilia and the long larval setae, all take place between 6- and 7-setigers. After this stage the larvae construct small tubes and feed on the bottom.

The development of the dorsal posterior tentacular cirrus from the first larval parapodium is described and figured. This cirrus is formed by the elongation of the lobe lying between the noto- and neuropodial setal bundles. It is not, therefore, strictly derived from the dorsal cirrus of the first parapodium.

The reproduction and development is discussed and shown to be very similar to that of the other members of the family. No special commensal adaptation was observed during the larval period. It is therefore concluded that this occurs after settlement.

REFERENCES

Banse, K., 1954. Über Morphologie und Larvalentwicklung von *Nereis* (*Neanthes*) *succinea* (Leuckart) 1847 (Polychaeta errantia). *Zool. Jb.*, Abt. 2, Bd. 74, pp. 160–71.

Bogucki, M., 1953. Rozród i rozwój wieloszczeta *Nereis diversicolor* (O. F. Müller) w Bałtyku. *Polsk. Arch. Hydrobiol.*, T. 1, pp. 251–70.

Charrier, H., 1921. Recherches sur la *Nereis fucata* Sav. *Act. Soc. linn. Bordeaux*, T. 72, pp. 5–144.

Dales, R. P., 1950. The reproduction and larval development of *Nereis diversicolor* O. F. Müller. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 321–60.

Davenport, D., 1955. Specificity and behaviour in symbioses. *Quart. Rev. Biol.*, Vol. 30, pp. 29–46.

DAVENPORT, D. & HICKOK, J. F., 1957. Notes on the early stages of the facultative commensal *Podarke pugettensis* Johnson (Polychaeta: Hesionidae). *Ann. Mag. nat. Hist.*, Ser. 12, Vol. 10, pp. 627–31.

DEFRETIN, R., 1949. Recherches sur la musculature des néréidiens au cours de l'épitoquie, les glandes parapodiales et la spermiogénèse. *Ann. Inst. océanogr. Monaco*, T. 24, pp. 117–257.

DURCHON, M., 1956. Mode de reproduction et développement de *Nereis* (*Ceratonereis*) *costae*, Grube; (Annelida, Polychète) à Alger. *Arch. Zool. exp. gén.*, T. 93, pp. 57–69.

FAGE, M. L., 1904. Sur la forme épitoque de *Nereis fucata* Sav. *Bull. Mus. Hist. nat., Paris*, T. 10, pp. 485–91.

FAUVEL, P. 1923. Polychètes errantes. *Faune Fr.*, No. 5, 488 pp. Paris.

FINKE, D., 1936. Zur Formbildung der Parapodien von *Nereis diversicolor* O. F. Müller. *Zool. Jb.*, Abt. 1 (Anat.), Bd. 61, pp. 239–342.

GREEN, J. & DALES, R. P., 1958. Biliverdin in the eggs of *Nereis fucata*. *Nature, Lond.*, Vol. 181, p. 1412.

HAUENSCHILD, C., 1951. Nachweis der sogenannten atoken Geschlechtsform des Polychaeten *Platynereis dumerilii* Aud. et M-Edw. als eigene Art auf Grund von Zuchtversuchen. *Zool. Jb.*, Abt. 3, Bd. 63, pp. 107–28.

HEMPELMANN, H., 1911. Zur Naturgeschichte von *Nereis dumerilii* Aud. et Edw. *Zoologica, Stuttgart*, Bd. 25, pp. 1–135.

HERPIN, R., 1923. Éthologie et développement de *Nereis* (*Neanthes*) *caudata* Delle Chiaje. *C.R. Acad. Sci., Paris*, T. 177, pp. 542–44.

—— 1926. Recherches biologiques sur la reproduction et le développement de quelques annélides polychètes. *Bull. Soc. Sci. nat. Ouest*, T. 5, pp. 1–250.

HORNELL, J., 1891. Report on the polychaetous annelids of the L.M.B.C. district. *Trans. Lpool biol. Soc.*, Vol. 5, pp. 223–68.

IZUKA, A., 1908. On the breeding habit and development of *Nereis japonica* n.sp. *Annot. zool. jap.*, Vol. 6, pp. 295–305.

JOHNSON, M. W., 1943. Studies on the life history of the marine annelid *Nereis vexillosa*. *Biol. Bull., Woods Hole*, Vol. 84, pp. 106–14.

JUST, E. E., 1914. Breeding habits of the heteronereis form of *Platynereis megalops* at Woods Hole, Mass. *Biol. Bull., Woods Hole*, Vol. 27, pp. 201–12.

—— 1922. On rearing sexually mature *Platynereis megalops* from eggs. *Amer. Nat.*, Vol. 57, pp. 471–78.

LANGERHANS, P., 1879. Die Wurmfauna Madeiras. II. *Z. wiss. Zool.*, Bd. 33, pp. 271–316.

LILLIE, F. R. & JUST, E. E., 1913. Breeding habits of the heteronereis form of *Nereis limbata* at Woods Hole, Mass. *Biol. Bull., Woods Hole*, Vol. 24, pp. 147–60.

MARE, M. F., 1942. A study of a marine benthic community with special reference to the micro-organisms. *J. mar. biol. Ass. U.K.*, Vol. 25, pp. 517–54.

MCINTOSH, W. C., 1910. *Monograph of the British Marine Annelids*, Vol. 2, Pt. 2, pp. 233–524. London: Ray Society.

REISH, D. J., 1954. The life history and ecology of the polychaetous annelid *Nereis grubei* (Kinberg). *Occ. Pap. Allan Hancock Fdn*, No. 14, pp. 1–46.

—— 1957. The life history of the polychaetous annelid *Neanthes caudata* (delle Chiaje), including a summary of development in the family Nereidae. *Pacif. Sci.*, Vol. 11, pp. 216–28.

RULLIER, F., 1954. Recherches sur la morphologie et la reproduction du néréidien *Micronereis variegata* Claparède. *Arch. Zool. exp. gén.*, T. 91, pp. 195–233.

SMITH, R. I., 1950. Embryonic development in the viviparous nereid polychaete, *Neanthes lightii* Hartman. *J. Morph.*, Vol. 87, pp. 417–66.

TAKAHASI, S., 1933. On the epitocous phase of the nereid, *Perinereis nuntia* var. *brevicirris* Grube. *Annot. zool. jap.*, Vol. 14, pp. 203–9.

WILSON, D. P., 1932. The development of *Nereis pelagica* Linnaeus. *J. mar. biol. Ass. U.K.*, Vol. 18, pp. 203–17.

WILSON, E. B., 1892. The cell-lineage of *Nereis*. *J. Morph.*, Vol. 6, pp. 361–480.

J. mar. biol. Ass. U.K. (1959) 38, 81–95
Printed in Great Britain

CARBOHYDRATE LEVELS IN *PATELLA*

By R. J. C. Barry
Department of Physiology, University of Sheffield

and K. A. Munday
Department of Physiology and Biochemistry, University of Southampton

(With Text-figs. 1–9)

The limited observations available on blood sugar levels of Mollusca suggest that they are very low. Values of 2–14 mg % glucose have been reported for *Aplysia* (see Kisch, 1929; Berthoumeyroux, 1935), and even the higher levels recorded for *Octopus* and *Sepia* species by Bierry & Giaja (1909), Berthoumeyroux (1935) and Derrien (1938) range only from 20 to 32 mg % glucose. The majority of these blood sugar analyses were based on the Hagedorn & Jensen (1923) technique. Landgrebe & Munday (1954) have shown that this technique may have an inherent blank error of 5 mg % glucose equivalent, and consequently it is not a suitable method for critical analyses of these low molluscan blood sugar levels.

Seasonal analyses of molluscan blood sugar levels have been restricted to the terrestrial *Helix pomatia* L. From monthly analyses, Schwarz (1935) could not detect any definite seasonal variation, beyond a possible increase before and at the end of hibernation. Wolf-Heidegger (1935) obtained higher mean blood sugar values (22 mg %) for summer as compared with hibernating *Helix* (11 mg %), but this seasonal difference was not confirmed by the investigations of Lustig, Ernst & Reuss (1937) and Holtz & von Brand (1940). It was desirable to extend these seasonal investigations to other Mollusca, using a method particularly suitable for small blood samples and low concentrations of glucose. The marine gastropod *Patella* was selected for these studies.

Rather more investigations have been carried out on polysaccharide reserves in Mollusca. Seasonal variation in the glycogen reserves of *Ostrea* species has been demonstrated by Mitchell (1915–16), Russell (1923) and Okazaki & Kobayashi (1929). From analyses of the individual tissues of *Gryphaea angulata* Lamk., Couteaux-Bargeton (1947) concluded that glycogen is used both during the overwintering period and in the formation of sexual products. The dual role of polysaccharides has been most clearly demonstrated in *Helix pomatia* (see von Brand, 1931; May, 1934), in which the two polysaccharides, glycogen and galactogen, have concentration maxima at different periods of the year. Glycogen is apparently used in the maintenance of metabolism during hibernation, with a maximum concentration at the beginning of this period. Galactogen, which is restricted to the albumen gland, forms the main polysaccharide reserve of the eggs, and has a maximum concentration at the onset of egg-laying.

The work reported in this paper records the seasonal levels of blood glucose in *Patella* paralleled by tissue glycogen concentrations. In addition, starvation effects on these carbohydrate levels have been established under laboratory conditions, and the effects of glucose administration on blood glucose concentration studied.

We wish to thank the Department of Scientific and Industrial Research for financial assistance to R.J.C.B. and thanks are also due to Dr B. J. R. Taylor for assistance in some field collections.

MATERIALS AND METHODS

Patella, used in these studies, occurred as a sheltered population between two natural rock barriers, which project southwards into the sea at Pevril Point, Swanage. For laboratory investigation they were transported packed in sea-weed and then maintained under starvation conditions in sea-water aquaria. Under these conditions, *Patella* readily re-attached themselves to a rocky substrate and could be maintained in a healthy condition for several months; the maximum period of survival recorded being 11 months.

In blood sampling, the head was pushed gently to the back of the head cavity, which was dried out with cotton-wool. Uncontaminated blood was withdrawn into a hypodermic syringe from the large vessel, which runs from the pallial vessel dorsally into the heart (Fig. 1). In the field, sampling was completed within 2 min of prising the limpet from its substrate. The blood was immediately mixed with tungstic acid protein precipitant and the deproteinised supernatant analysed on return to the laboratory.

The blood total reducing value, taken as an estimate of the blood glucose concentration, was determined by Landgrebe & Munday's (1954) modification of the Folin & Malmros (1929) technique. Duplicate analyses of 0·5 ml. *Patella* blood in 5 ml. tungstic acid gave values agreeing within 0·4 mg % glucose equivalent, confirming the suitability of the technique at these low levels.

Tissue glycogen concentrations were estimated by the Good, Kramer & Somogyi (1933) technique, the glycogen hydrolysate having been chromatographically identified as glucose. Tissues dissected from the living animal were weighed and immersed in hot KOH solution within 10 min of removing the animal from its shell. A homogeneous solution was obtained within 45 min of heating in a boiling water-bath, using a maximum of 200 mg wet weight of tissue per ml. 30 % KOH. The alcohol-precipitated glycogen was redissolved in 2 ml. 15% KOH and reprecipitated by adding 2 ml. absolute alcohol. After acid hydrolysis, the total reducing value was determined by the Landgrebe & Munday (1954) technique. Glycogen concentrations were expressed as % wet weight; that is, the weight of reducing substances, as g glucose, resulting from hydrolysis of the glycogen present in 100 g wet weight of tissue (glycogen as g glucose/100 g tissue).

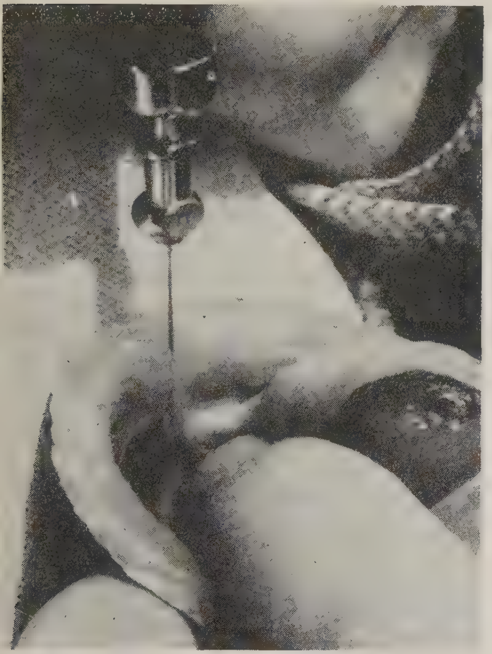


Fig. 1

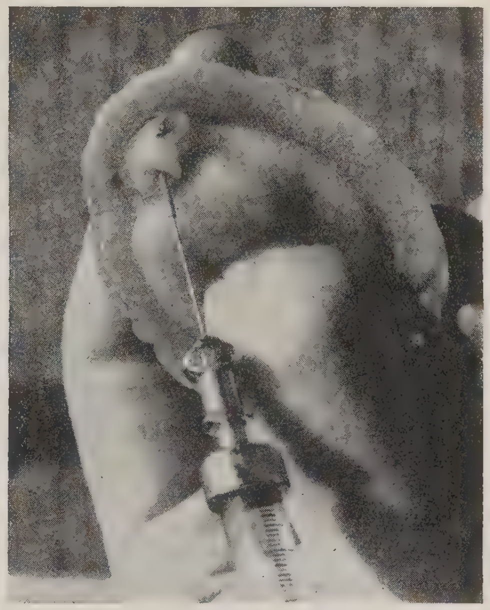


Fig. 2

Fig. 1. Methods of taking blood samples from *Patella*, showing the hypodermic needle in the vessel which runs dorsally from the pallial vessel into the heart.

Fig. 2. Method of oral administration of glucose solutions, after the tube has been passed down the gut into the stomach of *Patella*.

Glucose was administered intravascularly and orally. Glucose solutions were injected into the visceral sinus through the foot medianly from the ventral side. Oral glucose was administered by plastic tube (external diameter 1 mm and length 3 cm) passed over the buccal mass down the gut into the stomach (Fig. 2).

The three species of *Patella* present on the Swanage coast are not readily identified from their external characteristics, because of the considerable intergrading between species (Evans, 1953). Examination of the pleuricuspid teeth of a sample of *Patella* confirmed Evans's observation that *P. vulgata* L.

was the commonest species in sheltered habitats, such as exist at Pevril Point. All *Patella* used were of the same external appearance. Although most specimens were probably *P. vulgata*, this investigation has been assigned to the genus *Patella*, rather than to any one species.

RESULTS

BLOOD GLUCOSE CONCENTRATIONS

Seasonal variations (Fig. 3)

The blood glucose concentrations of field *Patella* were determined by analyses of blood samples taken in the field between February 1954 and July 1955. Limpets were taken from the full range of their vertical distribution and from different vertical faces of exposed rocks. No correlation was detected between the blood glucose level and the ecological position of individual *Patella*.

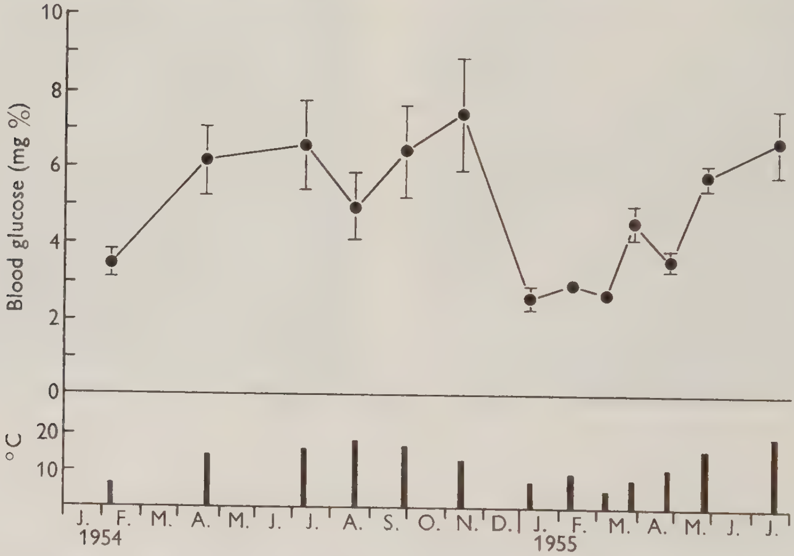


Fig. 3. Seasonal variation in blood glucose concentration of field *Patella*, showing the mean ± standard error of each series of analyses (10 individuals) and the sea temperature (shown below) at the time of blood sampling.

Between May and November, called the summer period, the mean glucose concentrations approximated to 6 mg % glucose. The means, with their standard errors, ranged from 4·9 ± 0·85 mg % to 7·3 ± 1·45 mg %, but this variation between means was not significant, because of the wide range of individual values in each series of analyses. A single very high value often accentuated the wide range in any particular series, the highest value recorded being 18·0 mg %. Between November and January, the mean blood glucose concentration decreased to the relatively low level of 2·5 ± 0·29 mg %. This low level persisted from January to the early part of March, with means ranging from 2·5 ± 0·29 mg % to 2·9 ± 0·12 mg %. This winter period was also characterized by the narrow range of individual values in each series of samples. The late-March and April series in 1955 gave values intermediate between the low winter level and the higher summer level, the latter having been re-attained in the May limpets (mean 5·7 ± 0·30 mg %).

The results indicate a definite seasonal variation of the blood glucose concentrations of *Patella* in their natural environment, the very variable summer values contrasting with the low relatively constant winter values.

Effect of starvation (Fig. 4)

Patella were maintained under starvation conditions in laboratory sea-water aquaria at a temperature similar to that occurring in the natural environment. In July 1954 the individual *Patella* sampled in the field (sea temperature 17° C) were brought into the laboratory and starved in sea-water aquaria at 17° C. After 4 days starvation, a second series of blood samples from these individual *Patella* was analysed. The mean blood glucose level of eight field *Patella* was 6·5 ± 0·84 mg %, with a range of 3·1–13·8 mg %. After 4 days starvation the mean blood glucose level had decreased to 4·9 ± 0·14 mg % and the range narrowed to 4·2–5·6 mg %. This latter level approximated to the lower values recorded in the July field samples. After this initial starvation effect, the blood glucose concentration decreased more gradually up to 40 days starvation (Fig. 4). Similar results were obtained with *Patella* collected in November.

The lower and more constant blood glucose concentrations of starved *Patella* suggest that the relatively high concentrations of some individual field *Patella* result from an alimentary hyperglycemia. On this hypothesis, the wide range of individual field values results from differences in the degree of this hyperglycemia and the lower values of starved *Patella* represent a post-absorptive blood glucose level.

During the winter period, the low and relatively constant glucose concentrations of field *Patella* suggest that they do not feed during this period. This was supported by starvation experiments, in which January *Patella* were maintained in sea-water aquaria at the field sea temperature of 6° C. After 11 days starvation, the mean blood glucose concentration was 2·9 ± 0·24 mg %, as compared with 2·5 ± 0·29 mg % for the field analyses. In contrast to the summer *Patella*, the blood glucose had not decreased on starvation, indicating that it was already at a basal level in field *Patella* during the winter period. That the blood glucose concentration of January field *Patella* collected at 6° C could decrease was shown by raising the aquarium temperature to 17° C. From a mean field value of 2·5 ± 0·29 at 6° C, the mean blood glucose concentration after 5 days starvation at 17° C was 1·9 ± 0·36 mg %, and after

17 days starvation at 17° C 1·6 ± 0·13 mg %. The higher aquarium temperature at which these January field *Patella* were maintained may have caused an increased utilization of available carbohydrate. The results suggest that the disappearance of glucose from January starved *Patella* is temperature dependent.

Yeast fermentation experiments suggested that the true blood glucose concentrations of starved *Patella* were even lower than the total reducing values determined by the Landgrebe & Munday technique. In July *Patella* starved for 4 days in the laboratory, the true blood glucose concentrations approximated to 40 % of the total reducing values. The decrease in the total reducing value of winter *Patella* starved at 17° C appeared to result from a decrease in the true blood glucose concentration. The marked individual variation of total reducing value of summer *Patella* also apparently represents a real variation in true blood sugar level. However, the yeast fermentation technique at these low glucose concentrations was liable to considerable error, and consequently this technique was not applied as a routine procedure to all blood samples.

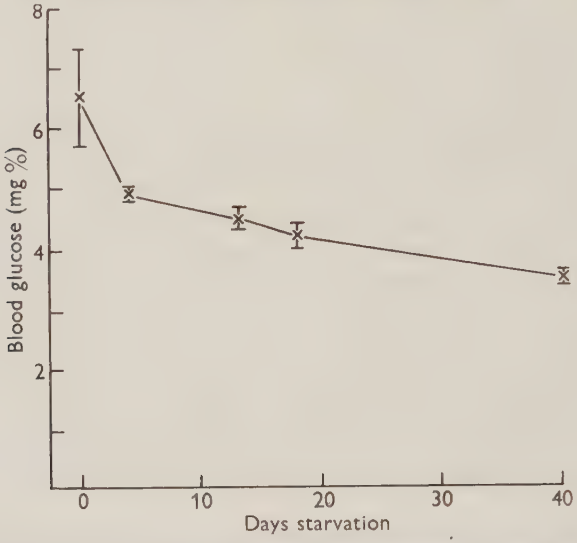


Fig. 4. Effect of starvation on the blood glucose concentration of *Patella*, showing the mean ± standard error of each series of analyses (8 individuals). July *Patella* collected at sea temperature of 17° C and maintained at that temperature in the aquaria.

Glucose administration experiments (Fig. 5)

The disappearance of glucose from the blood was investigated by injection experiments. 2 mg glucose in 0·2 ml. sea water was injected into the visceral sinus of starved summer *Patella* maintained at 17° C, and a hyperglycemia of approximately 30 mg % was recorded 10 min after injection (Fig. 5). Within 4 h the blood glucose concentration had returned to the pre-injection level of 2·5 mg %. Injection of the same volume of sea water alone had no hyperglycemic effect. The sea water surrounding the limpet was analysed for glucose, in order to establish whether any glucose was being excreted during the experiment. Even after injection of up to 15 mg glucose, which induced a hyperglycemia of over 200 mg %, less than 2 % of the total glucose injected could be recovered from the sea water. Thus, there was no appreciable escape of glucose through the body surface of the limpet, nor was the injected glucose excreted by the kidney during the period of the experiment.

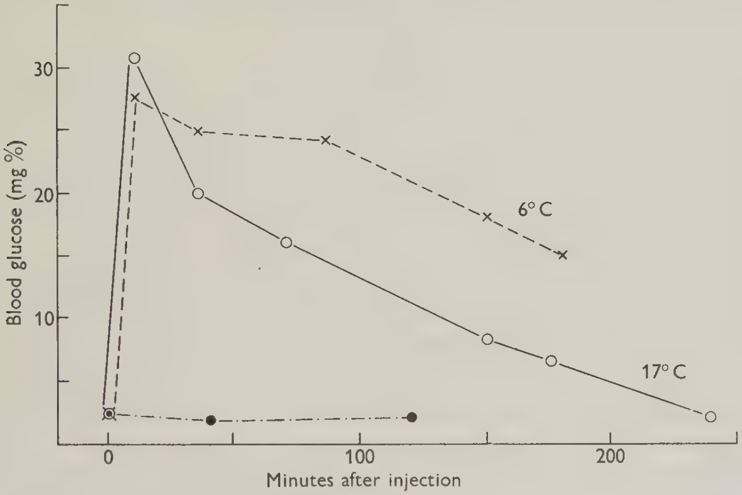


Fig. 5. Variation of blood glucose concentration of *Patella*: (○——○), after the intravascular injection of glucose solution at 17° C, mean 8 individuals; (× - - - - - ×), glucose solution at 6° C, mean 6 individuals; and (●–·–·–●), of sea water, mean 8 individuals, showing mean blood glucose values with increasing periods of time.

This rapid removal of glucose from the blood probably resulted from its metabolism to a non-reducing product. Using starved winter *Patella* maintained at 6° C, the rate of disappearance of injected glucose from the blood was considerably slower than in the summer *Patella* at 17° C. This also suggested that some active metabolic process was involved in the removal of glucose.

Similar results were obtained from April and May *Patella*, to which glucose was administered orally. Oral administration of small volumes of sea water had no effect on the blood glucose concentration, but oral administration of 0·3 ml. 1 % glucose/sea-water solution induced a hyperglycemia of approximately 30 mg % within 15 min. This suggested a rapid transfer of glucose from the alimentary canal to the blood stream. In animals at 17° C, the initial glucose levels were re-attained within 4 h, but animals maintained at 6° C still possessed elevated blood glucose levels 5 h after oral administration.

TISSUE GLYCOGEN CONCENTRATIONS

Seasonal variations (Fig. 6)

The hepatopancreas (including the intestine), the odontophore cushion and the foot were shown to be the main glycogen storage tissues of *Patella*, each normally having a glycogen concentration greater than 1 % wet weight during the summer months. In contrast, the glycogen concentrations of the gonad, the mantle skirt and the remainder of the head region never exceeded 1 % wet weight.

Seasonal determinations of the glycogen concentrations of the three main storage tissues were carried out during the period July 1954 to July 1955, and paralleled the seasonal investigations of the blood glucose concentrations. Analyses were commenced within 48 h of bringing *Patella* into the laboratory, in order to minimize starvation effects, although experiments indicated no significant change in glycogen concentrations up to 14 days after field collection.

The hepatopancreas glycogen concentration exhibited a well defined seasonal variation (Fig. 6A). From July to November the mean values approximated to 2 % wet weight of tissue, but each series of analyses contained a wide range of individual values. The glycogen concentrations decreased sharply between November and January, and this was followed by a winter period characterized by low relatively constant means of approximately 0·3 % wet weight and each series showing little individual variation. This winter period persisted from January to the early part of March. Intermediate values in late March and April constituted a spring rise in hepatopancreas glycogen concentration, the summer level of the previous year being re-attained in the May limpets.

The glycogen concentration of the odontophore cushion did not exhibit a marked seasonal pattern (Fig. 6B). Very variable values were obtained throughout the year. Following a sharp decrease in the mean value between November and January, low mean glycogen concentrations did not persist in the February and March series, although the majority of the individual values of < 1 % wet weight occurred during this period.

Foot glycogen concentrations were determined by analyses of pieces of tissue from the mid-foot region (Fig. 6C). The general pattern of the seasonal variation resembled that of the hepatopancreas. During July to November, the foot glycogen concentrations of individual *Patella* exhibited considerable variations, and the mean level tended to decrease in the October and November series. In January, the glycogen concentration had fallen to a low winter level of approximately 0·8 % wet weight, a somewhat higher level than that recorded in the hepatopancreas (0·3 % wet weight). The hepatopancreas also differed from the mid-foot, in that the spring rise in its glycogen concentration preceded that of the mid-foot. The low winter level of mid-foot glycogen persisted until April and the spring rise was not evident before the May

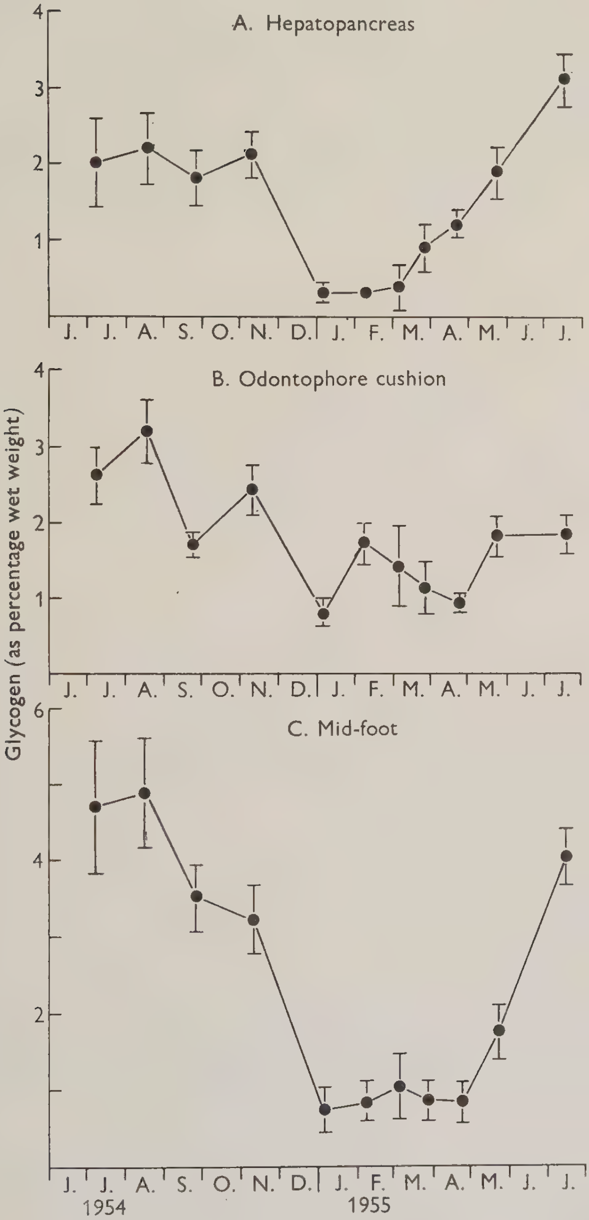


Fig. 6. Seasonal variations in tissue glycogen concentrations of (A) the hepatopancreas, (B) the odontophore cushion, and (C) the mid-foot of *Patella*, showing the mean ± standard error of each series of analyses (7 individuals).

analyses, by which time the hepatopancreas glycogen concentration had already re-attained the summer level of the previous year.

Relative weights of tissues (Fig. 7)

The tissue difference in the spring rise of glycogen concentrations was accentuated when the relative weights of *Patella* tissues were considered. The weights of the foot and of the remaining tissue after dissecting away the gonad were recorded. The bulk of this remaining tissue consisted of hepatopancreas, and consequently variation in the ratio remaining-tissue-weight/foot-weight probably indicated a variation in the weight of the hepatopancreas relative to the foot. This ratio increased sharply in March and April, but returned to the previous level in the May limpets (Fig. 7).

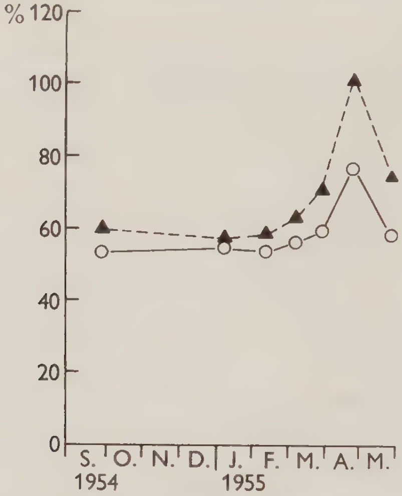


Fig. 7. Seasonal variations in weight of 'remaining' tissues, shown as a percentage of the weight of the foot, giving the mean values of 8 individuals calculated from wet weights (○) and dry weights (▲) of tissues. 'Remaining' tissues are the soft tissues of *Patella* after dissecting away the gonad and the foot.

This increase in the ratio coincided with the spring rise in hepatopancreas glycogen concentration, but could not be accounted for solely by an increased weight due to glycogen storage. It seems probable that there was an actual growth of the hepatopancreas relative to the foot. If so, the increase in the total glycogen reserves of the hepatopancreas during the spring rise is even greater than that indicated by the increase in glycogen concentration.

Effect of starvation (Fig. 8)

Glycogen concentrations of the main storage tissues were determined with increasing periods of starvation. *Patella*, collected in July, were maintained in sea-water aquaria at the July sea temperature of 17° C. During the first month's starvation there was no marked decrease in the tissue glycogen concentrations. After 60 days starvation, the concentration of the hepatopancreas (0·4% wet weight) had decreased to the winter level. The concentrations of the odontophore cushion and mid-foot had also fallen, but they still contained appreciable glycogen concentrations with mean values 1·4 and 1·5% wet weight respectively. On prolonged starvation of 120 days, the glycogen concentrations of all tissues were extremely low, no individual value exceeding 0·4% wet weight.

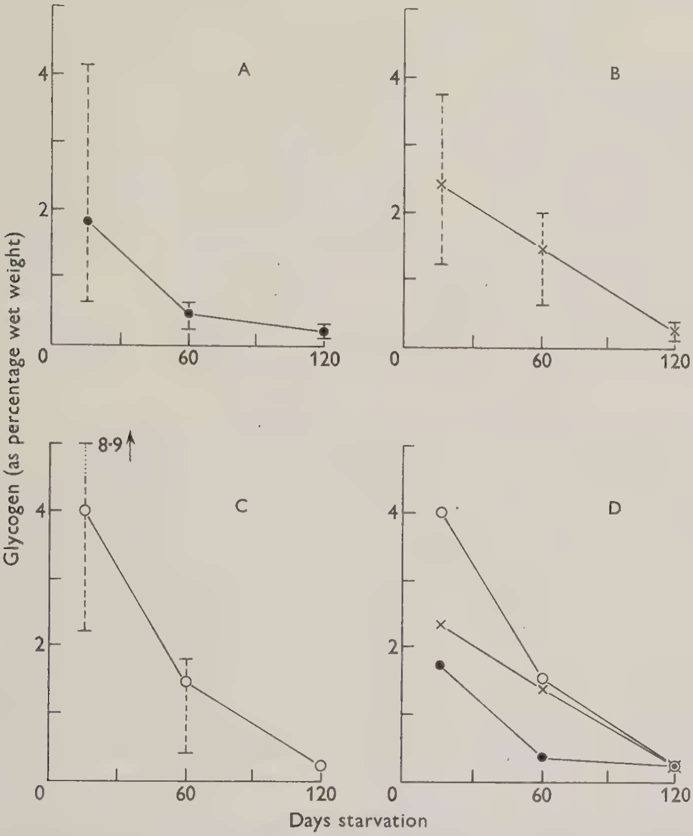


Fig. 8. Variations in tissue glycogen concentrations of *Patella* collected in July and maintained in sea-water aquaria at 17° C. Graphs A, B and C show the means and range of individual values (12 for 5 days, 4 for 60 and 4 for 120 days) with increasing periods of starvation for the hepatopancreas (●), odontophore cushion (×) and the mid-foot (○) respectively. Graph D shows the mean values for the three tissues superimposed.

Well-developed gonads were present in *Patella* starved for 60 and 120 days. This gonadial development paralleled that occurring in the natural population over the same period (August–November). The decrease in the tissue glycogen reserves of starved *Patella* may have resulted from glycogen utilization both in maintaining metabolism and in the development of the gonads. However, in starved *Patella* the glycogen concentrations of the gonads themselves did not exceed 0·1 % wet weight.

RESERVE MATERIALS IN THE GONAD OF *PATELLA*

During gonadial development in the field population (August–November), the gonad glycogen concentration never exceeded 1 % wet weight. The highest glycogen concentrations (mean 0·6 % wet weight) occurred during early development in August, fully developed gonads in November having a mean concentration of 0·2 % wet weight. Smears of developing gonads stained black with osmic acid and 30–36 % dry weight of the August gonads could be extracted in a soxhlet by petroleum ether (boiling range 40–60° C). This represented 10–12 % wet weight of the gonad. This extracted material was very oily and probably contained a high percentage of fat. Therefore, fat may form a major reserve material in the development of the sexual products.

DISCUSSION

A seasonal variation exists in the blood glucose concentration of *Patella*. The wide range of individual values of the summer period, with means approximating to 6 mg %, contrasted with the lower and more constant values of the winter period, giving means of approximately 3 mg %. Even the higher mean blood glucose levels of summer *Patella* were lower than those previously reported for other more active molluscan species, e.g. *Helix* and Cephalopoda (8–33 mg %). This may have resulted from the difficulty in the measurement of very low blood glucose levels using the techniques previously employed. Alternatively, it could be indicative of a correlation between the blood glucose level and activity in molluscs, similar to that suggested in fishes (Gray & Hall, 1930).

The decrease in the blood glucose level on starvation of summer *Patella* suggests that the higher concentrations in individual field *Patella* result from an alimentary hyperglycemia. The similarity between the low concentrations of both field and starved *Patella* during the winter months suggests that *Patella* do not feed during this winter period, and that they may pass into an inactive state comparable to the hibernation of terrestrial molluscs.

A close correlation exists between the seasonal variations of the blood glucose and hepatopancreas glycogen concentrations (Fig. 9). Between November and January, these concentrations decreased to a relatively low and constant winter level, which persisted into the early part of March. The spring rise in the concentration of the hepatopancreas glycogen also paralleled that of the blood glucose, but preceded the increase in foot glycogen concentration.

The total increase in the glycogen reserves of the hepatopancreas was probably even greater than indicated by the glycogen concentration alone, because of an actual growth of the hepatopancreas relative to the foot (March–April). This close parallel between the spring rise in blood glucose and hepatopancreas glycogen concentration may be associated with the function of the hepatopancreas as a digestive and absorptive organ and the recommencement of feeding. It is not suggested, however, that there is any functional analogy with the vertebrate liver as the main glycogen storage organ.

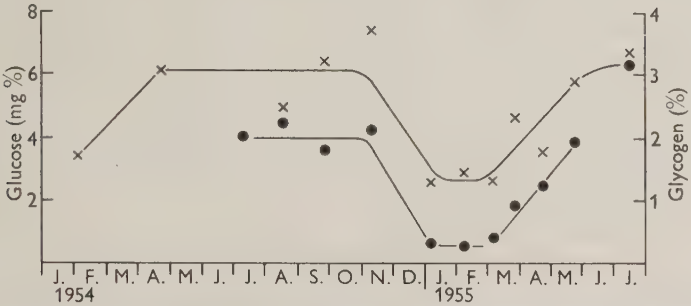


Fig. 9. Patterns of seasonal variations in blood glucose concentrations (upper line, ×) and hepatopancreas glycogen concentrations (lower line, ●) of field *Patella*.

The decrease in glycogen concentrations of all tissues of *Patella* between November and January coincided with the shedding of the genital products. The resultant low levels of hepatopancreas and foot glycogen remained constant throughout the winter period, no further decrease being detected before the spring rise commenced at the end of March. In the decrease of glycogen reserves with the shedding of the genital products, the seasonal pattern of tissue polysaccharide reserves in *Patella* resembled that reported for *Helix* (von Brand, 1931; May, 1934) and oysters (Couteaux-Bargeton, 1947). But in these latter species, this decrease was followed by a period of accumulation before the onset of the winter period, during which there was a second decrease in the polysaccharide reserves. It has been suggested that in *Helix* and oysters glycogen is utilized in maintaining the metabolism during the winter period. In *Patella* the absence of changes in the low levels of glycogen in the hepatopancreas and foot between January and March offer no evidence of a similar glycogen utilization.

In field *Patella* gonadial development occurred between August and November. At no period of the year did the gonad contain appreciable glycogen reserves, not even preceding sexual maturity. This contrasts with the large reserves of galactogen laid down in the albumen gland of *Helix* (May, 1934) and of glycogen in the gonad of oysters (Couteaux-Bargeton, 1947) prior to sexual activity. However, petroleum ether extracts of developing gonads of *Patella* suggest that fat rather than polysaccharide forms a major reserve material in the sexual products, in a manner somewhat similar to the storage of fat rather than glycogen in the digestive gland of *Pila* (George & Desai, 1954). Nevertheless, the rapid decrease in glycogen reserves between November and January is unlikely to result solely from the maintenance of metabolism after the cessation of feeding, since short-term starvation (1 month) at a much higher temperature (+10° C) does not result in a rapid utilization of glycogen. Glycogen may be used up rapidly in the final stages of gonadial maturation.

During sexual development in the field, the tissue glycogen concentrations do not markedly decrease. In contrast, gonadial development still occurs in laboratory-starved *Patella*, but is accompanied by a considerable decrease in glycogen concentrations. These results suggest that the alimentary carbohydrate recorded in the field *Patella* might be exerting a 'glycogen-sparing effect' during gonadial maturation, whereas in starved *Patella* the glycogen reserves may be transformed into fat.

SUMMARY

Blood glucose concentrations (total reducing values) of field *Patella* showed a seasonal variation. The variable summer values, whose means approximated to 6 mg % glucose (May–November), contrasted with the relatively low and constant winter values, whose means approximated to 3 mg % glucose (January–March). On starvation of summer *Patella*, the higher field values rapidly decreased to a constant basal level, more comparable to the low winter field values. A hyperglycemia of 30 mg % induced by intravascular glucose injection was followed by a rapid adjustment of the blood glucose to the pre-injection level within 4 h.

Seasonal variations in the glycogen concentrations occurred in the hepatopancreas and foot, but were not evident in the odontophore cushion, the other main site of glycogen storage. The patterns of the seasonal glycogen variations were similar to those of blood glucose, the parallelism being closer in the hepatopancreas than in the foot glycogen changes. As with the blood glucose, the spring rise in hepatopancreas glycogen concentration occurred during March and April, and was accompanied by a growth of the hepatopancreas. The spring rise in the foot glycogen concentration did not commence until May.

The developing gonads of both field and starved *Patella* (August–November) contained no appreciable glycogen reserves, but petroleum ether extracts indicated considerable fat storage. The shedding of the genital products coincided with the sharp decrease in blood glucose and tissue glycogen concentrations (November–January). Unlike oysters, the glycogen levels remained low throughout the winter period.

REFERENCES

Berthoumeyroux, M., 1935. Recherches sur le pouvoir réducteur des liquides du milieu intérieur de quelques invertébrés marins. *Bull. Soc. sci. Arcachon*, T. 32, pp. 111–53.

Bierry, H. & Giaja, J., 1909. Dosage du sucre du sang chez le poulpe (*Octopus vulgaris* L.). *C.R. Soc. Biol., Paris*, T. 66, pp. 579–80.

Brand, Th. von, 1931. Der Jahreszyklus im Stoffbestand der Weinbergschnecke (*Helix pomatia*). *Z. vergl. Physiol.*, Bd. 14, pp. 200–64.

Couteaux-Bargeton, M. 1947. Variations du taux de glycogène dans différents organes de l'huître. *Bull. Soc. zool. Fr.*, T. 71, pp. 121–6.

Derrien, Y., 1938. Répartition du chlorure de sodium et du glucose entre le plasma et le corps vitré chez *Octopus vulgaris* Lmk. et *Sepia officinalis* L. *C.R. Soc. Biol., Paris*, T. 127, pp. 1011–14.

Evans, R. G., 1953. Studies on the biology of British limpets—the genus *Patella* on the south coast of England. *Proc. zool. Soc. Lond.*, Vol. 123, pp. 357–76.

Folin, O. & Malmros, H., 1929. An improved form of Folin's micro method for blood sugar determinations. *J. biol. Chem.*, Vol. 83, pp. 115–20.

George, J. C. & Desai, B. N., 1954. On the liver fat in *Pila globosa* (Swainson). *J. Anim. Morph. Physiol.*, Vol. 1, pp. 56–8.

Good, C. A., Kramer, H. & Somogyi, M., 1933. The determination of glycogen. *J. biol. Chem.*, Vol. 100, pp. 485–91.

Gray, I. E. & Hall, F. G., 1930. Blood sugar and activity in fishes with notes on the action of insulin. *Biol. Bull., Woods Hole*, Vol. 58, pp. 217–23.

Hagedorn, H. C. & Jensen, B. N., 1923. Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. *Biochem. Z.*, Bd. 135, pp. 46–58.

Holtz, F. & Brand, T. von, 1940. Quantitative studies upon some blood constituents of *Helix pomatia*. *Biol. Bull., Woods Hole*, Vol. 79, pp. 423–31.

Kisch, B., 1929. Der Gehalt des Blutes einiger Wirbelloser an reduzierenden Substanzen. *Biochem. Z.*, Bd. 211, pp. 292–4.

Landgrebe, F. W. & Munday, K. A., 1954. A modified Folin-Malmros technique for the estimation of blood sugar, particularly suitable for low values or for micro work. *Quart. J. exp. Physiol.*, Vol. 39, pp. 17–22.

Lustig, B., Ernst, T. & Reuss, E., 1937. Die Zusammensetzung des Blutes von *Helix pomatia* bei Sommer- und Wintertieren. *Biochem Z.*, Bd. 290, pp. 95–8.

May, F., 1934. Chemische und biologische Untersuchungen über Galaktogen. (Biologischer Teil: Der Jahreszyklus im Galaktogen- und Glykogenbestand der Weinbergschnecke.) *Z. Biol.*, Bd. 95, pp. 401–30.

Mitchell, P. H., 1915–16. Nutrition of oysters: glycogen formation and storage. *Bull. U.S. Bur. Fish.*, Vol. 35, pp. 151–62.

Okazaki, K. & Kobayashi, S., 1929. The seasonal variation of the glycogen content in the oyster, *Ostrea circumpicta* Pils. *Sci. Rep. Tôhoku Univ.*, Ser. D, Vol. 4, pp. 183–91.

Russell, E. S., 1923. Report on seasonal variation in the chemical composition of oysters. *Fish. Invest., Lond.*, Ser. 2, Vol. 6, No. 1.

Schwarz, K., 1935. Über den Blutzucker der Weinbergschnecke (*Helix pomatia* L.). *Biochem. Z.*, Bd. 275, pp. 262–9.

Wolf-Heidegger, G., 1935. Der Kohlenhydratstoffwechsel der Weinbergschnecke (*Helix pomatia* L.) unter der Einwirkung von Insulin und Adrenalin. *Biochem. Z.*, Bd. 279, pp. 55–63.

J. mar. biol. Ass. U.K. (1959), 97–102
Printed in Great Britain

ON THE METAMORPHOSIS OF THE VISUAL PIGMENTS OF *ANGUILLA ANGUILLA* (L.)

By D. B. Carlisle and E. J. Denton
The Plymouth Laboratory

(Text-fig. 1)

The retinae of all the species of deep-sea fish which have been studied have been shown to contain golden coloured pigments, chrysopsins, which are especially suited to the light found in their natural environment (Denton & Warren, 1957; Munz, 1958). Amongst fish caught in shallow coastal waters this characteristic colour of retinal pigment is found only in the conger eel *Conger conger* (by Denton & Walker, 1958), and amongst freshwater fish only in the silver eel *Anguilla anguilla* (by Carlisle & Denton, 1957, referred to by Denton & Warren, 1957). Both these fish are species of Apodes, a group of which the vast majority of species are deep-water forms. Both species, moreover, begin their life in the deep sea and return to it again when mature to spawn and may therefore, in one sense, be regarded as deep-sea fish. Since immature conger eels in shallow coastal waters already have a retinal pigment characteristic of a deep-sea fish, it seems very unlikely that on returning to deep water they would change away from the deep-sea form which they already possess. It is probable therefore that the conger retains a deep-sea form of retinal pigment throughout the whole of its life.

There remained, on beginning these experiments, a curious difference between the silver form of the European eel, *A. anguilla*, with its golden coloured retina, and the North American eel, *A. rostrata*, which had been shown to have a reddish purple retina believed to contain a mixture of rhodopsin and porphyropsin (Wald, 1945). It appeared then that either there was a difference between these two species of the genus *Anguilla*, or that there was a change of pigments during the life of the freshwater eel similar to that known to take place when the tadpole of the bullfrog becomes adult (Wald, 1945).

The larva of *A. anguilla* is a leptocephalus, which metamorphoses to a juvenile elver as it approaches the coasts of Europe. The juvenile eel spends some years in the estuaries and rivers of Europe, where it is known as the yellow eel; a second metamorphosis then begins during which the eel assumes a silvery dress and the gonads begin to grow; this silver eel then migrates towards the deep Atlantic spawning grounds. This change of livery once begun can be hastened by injections of gonadotrophic hormones. Associated with the change in livery is an enlargement of the eye. D'Ancona (1927,

1929) and Ferriani (1954) have studied this increase in the size of the eye and have shown that the diameter is doubled and the retinal area increases fourfold. D'Ancona states that despite this increase in area the number of rods in the retina remains constant. From this Bertin (1951; English edition, 1956) argues that as the eye enlarges the retina '...must be less and less capable of light perception and that, on the whole, the enlargement of the eye constitutes degeneration rather than improvement' (English edition, 1956, p. 96).

We therefore posed ourselves two questions:

(1) Is there a change of retinal pigment at the change of livery in *A. anguilla*?

(2) Is the enlargement of the eye at this change of livery accompanied by any decrease in efficiency of the eye in absorbing light?

MATERIAL AND METHODS

The eels were all caught in fresh water; the deep-sea eel was caught in an Agassiz trawl and its retina was studied at sea aboard R.V. 'Sarsia'.

The retinae, from the eyes of eels which had previously been dark adapted for 2 days, were dissected out under a dim red light, mounted in a small chamber on a microscope slide in Ringer solution (Denton & Wyllie, 1955), and studied whilst fresh.

The spectral absorption of light by the retina was measured by methods described in detail by Denton & Walker (1958). Using one method (Method III of Denton & Walker) the total optical density[1] of the retina relative to an equal thickness of Ringer solution is determined as a function of wavelength; using another method (Method II of Denton & Walker) the difference in density between unbleached retina and the same retina after bleaching with strong white light is determined as a function of wavelength.

In addition to the normal yellow and silver eels eight of the latter which had been injected with gonadotrophins to advance the degree of sexual maturity were used. Each of these animals was injected weekly for 4 weeks with 500 international units of human chorionic gonadotrophin and the same amount of mare serum gonadotrophin. Of these animals one of the males reached sexual maturity, while the females all showed enlarged ovaries and ova up to 400 μ diameter. The eyes were further enlarged, but the retinae showed no significant differences from the normal silver eels and will not be considered further.

For histological purposes excised eyes were injected with Zenker's fixative and then fixed whole in this fluid, embedded in celloidin, sectioned and stained with Heidenhain's iron haematoxylin and van Gieson's stain.

---

[1] The densities referred to in this communication are all optical densities:

$$\log_{10}\left(\frac{\text{light incident}}{\text{light transmitted}}\right).$$

RESULTS

Several retinae of silver and yellow eels were dissected in quick succession and examined side by side in white light; the silver eel's retinae were golden in colour whilst those of the yellow eel were purple coloured. The density of pigment was seen to be at least as high if not higher in the silver eels than in the yellow eels. These simple observations confirmed observations made a year earlier on the silver eel and showed that there was a metamorphosis of the fresh water eel's visual pigments. Fig. 1 A shows that on metamorphosis of the visual pigment there is a shift in the maximum of the retinal difference curve of about 33 mμ and Fig. 2 B shows that the mature silver eel has a curve of total retinal density very close to that of the conger eel. The metamorphosis is accompanied by an increase in retinal density. The maximum retinal density changes on bleaching two specimens of yellow eel were 0·34 and 0·21, whilst the corresponding changes for the silver eel were 0·58 and 0·52.

A specimen of a deep-sea eel *Synaphobranchus* sp. caught in an Agassiz trawl at a depth of about 800 m off the north coast of Spain gave a total retinal density curve very close to those of the conger eel and the silver fresh-water eels.

The change in the eye as the eel changes from yellow to silver livery is well marked. In a specimen of yellow eel 61 cm long the longest diameter of the eyeball was 3·3 mm while in a silver eel of the same length which had been injected with gonadotrophins this diameter was 7·8 mm. The relative thickness of the various layers of the retina was slightly altered, but we have not sectioned enough specimens to be certain that this change is constant. Most layers are thinner in the silver eel. The number of rods in a median section of these two retinae is approximately the same. They are no wider spaced in the silver eel, however, for the external segments of the rods are about twice the diameter of the rods in the yellow eel (3 μ and 1·5 μ respectively). This enlargement in the rods seems adequate to account for the greater area of the retina. The rods are not increased significantly in length but we cannot find that they become shorter, as implied by D'Ancona (1927), who states that all the layers of the retina become distinctly thinner, though this is least marked in the layer of rods. ('I vari strati retinici sono distintamente assottigliati. Tale fatto è invece meno accentuato nello strato dei bastoncelli.') In comparable regions of the retina the thicknesses of the rod layer, measured from the external limiting membrane to the pigmented layer, were 92 μ in a silver eel and 86 μ in a yellow eel at the thickest part.

DISCUSSION

During the metamorphosis from the immature yellow to the mature silver form there are extensive changes equipping the eel for its deep-sea environment. These results show that these changes include one in which the retinal pigment becomes similar to those of the deep-sea fish. This takes place even before the fish leaves fresh water. The change is analogous to that described 14 years ago in the bullfrog (Wald, 1945), and to the change in retinal pigments recently shown to take place in the lamprey (Wald, 1957). Wald, Brown & Brown (private communication) have now investigated the chemistry of the metamorphosis of the visual pigment in the freshwater eel and have not only confirmed these results but have elucidated in detail both the nature and

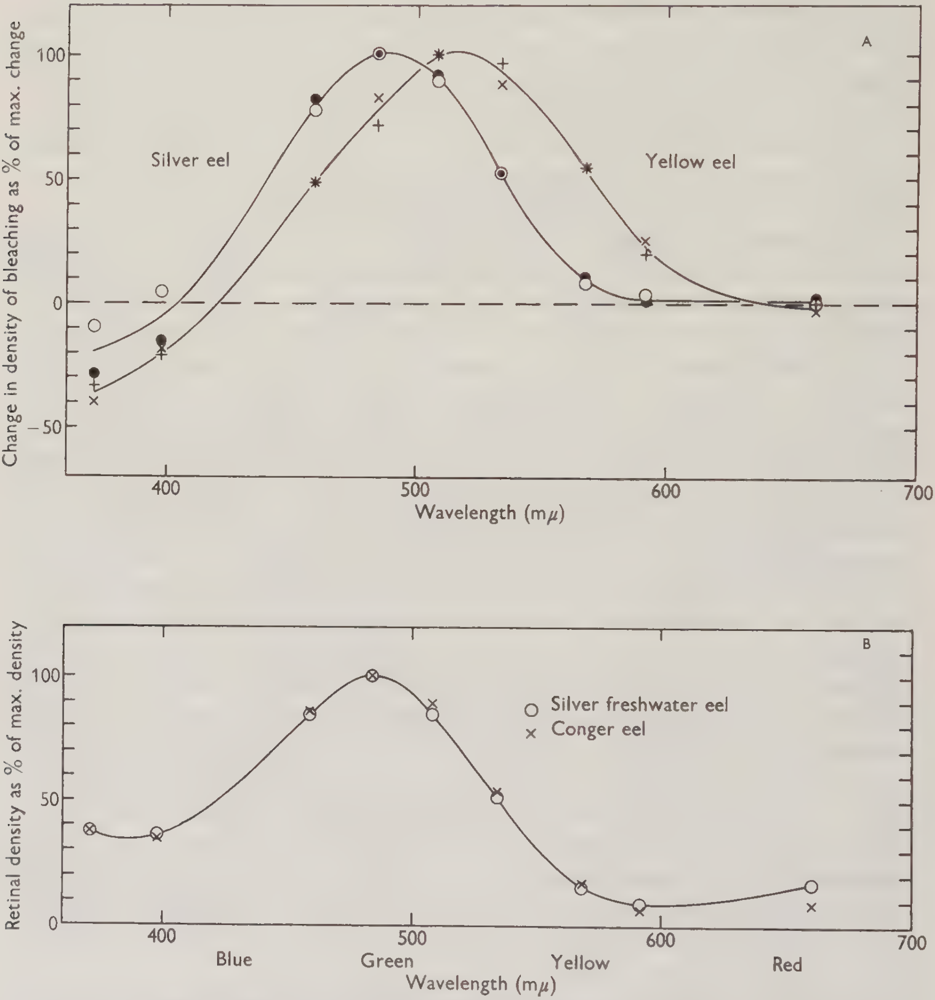


Fig. 1. A, *Anguilla anguilla*: immature (yellow) eels and mature (silver) eels. Spectral curves of difference in density between unbleached dark adapted retinae and the same retinae an hour after bleaching their photosensitive pigments with strong white light. B, *Conger conger* and mature (silver) eels (*Anguilla anguilla*). Spectral curves of densities of unbleached retinae with respect to an equal thickness of Ringer's solution.

course of this metamorphosis. Their results and those obtained here are in good accord.

Whilst the observations of D'Ancona on the histology of the eyes of yellow and silver eels are confirmed here, the deductions made from D'Ancona's observations by Bertin would seem to be false. The fraction of light incident on the retina absorbed by the retinal pigments is very high in the silver eel; after taking account of the shift in absorption curve the eye of the silver eel is more efficient than that of the yellow eel in usefully absorbing light striking the retina.

Despite the great change in calcium metabolism there is no change in the absorption of light by the crystalline lens on metamorphosis (Denton, E. J. & Warren, F. J. unpublished observation), but in size the lens and the pupil increase even more than does the eye itself.

For a silver and a yellow eel looking at the same small source of light at the same distance, the total light thrown on to the silver eel's retina will be greater (because of the *absolutely* larger pupil), the brightness of the silver eel's retinal image will be greater (because of the *relatively* larger pupil), and the fraction of light absorbed by the retinal photosensitive pigments will be greater (because of the higher retinal density of pigment).

We are very grateful to Mr A. C. G. Best for making the histological preparations for us.

We are indebted to the Royal Society for a scientific grant-in-aid, and to Organon Laboratories Limited for a gift of gonadotrophic hormones.

SUMMARY

European eels, *Anguilla anguilla* (L), while they retain the immature 'yellow' livery, have purplish coloured retinae, close in colour to those of freshwater fish. The purple colour is that of photosensitive pigments.

When an eel assumes the 'silver' livery of approaching maturity, in preparation for the deep-sea transatlantic migration to the breeding grounds off the Bermudas, the eye enlarges and the retina changes to a golden colour, like that of the photosensitive chrysopsins of deep-sea fish. The maximum of absorption shifts by about 33 mμ towards the shorter wavelength end of the spectrum and the final total retinal density curve of the silver eel is almost identical with that of the conger eel, *Conger conger* (L), and of a deep-sea eel, *Synaphobranchus* sp. This change of pigment takes place even before the fish leaves fresh water.

The larger eye and the relatively even larger pupil of the silver eel make it a more efficient light-collecting organ, whilst the total retinal density of photosensitive pigment is higher in the silver than the yellow eel and the retina will therefore be more efficient in usefully absorbing the light incident on it.

REFERENCES

BERTIN, L., 1951. *Les Anguilles*. Paris.

—— 1956. *Eels*. London.

CARLISLE, D. B. & DENTON, E. J., 1957. A change in visual pigments in the life of the fresh water eel. *J. Physiol.*, Vol. 139, p. 8P.

D'ANCONA, U., 1927. Richerche sull'ingrandimento dell'occhio dell'anguilla in rapporto alla maturità sessuale e considerazioni sul suo significata biologico. *R.C. Accad. Lincei*, Vol. 5, Ser. 6, pp. 360–3.

—— 1929. Sui fenomeni di circolazione che accompagnano l'ingrandimento dell'occhio nella maturità sessuale dell'*Anguilla*. *Boll. Soc. eustach.*, Anno 27 (1–2), No. 7, pp. 1–8.

DENTON, E. J. & WALKER, MARY ANN, 1958. The visual pigment of the conger eel. *Proc. roy Soc.* B, Vol. 148, pp. 257–69.

DENTON, E. J. & WARREN, F. J., 1957. The photosensitive pigments in the retinae of deep sea fish. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 651–62.

DENTON, E. J. & WYLLIE, J. H., 1955. Study of the photosensitive pigments in the pink and green rods of the frog. *J. Physiol.* Vol. 127, pp. 81–9.

FERIANI, M., 1954. Variazioni morfologici e funzionali dell'occhio dell'*Anguilla* nell'acquisizione dell'abito riproduttivo. *Boll. Zool.* Vol. 21, pp. 401–4.

MUNZ, F. W. 1958. Photosensitive pigments from retinae of certain deep-sea fishes. *J. Physiol.*, Vol. 140, pp. 220–35.

WALD, G., 1945. The chemical evolution of vision. *Harvey Lect.*, Vol. 41, pp. 117–60.

—— 1957. The metamorphosis of the visual systems in the sea lamprey. *J. gen. Physiol.*, Vol. 40, pp. 901–14.

J. mar. biol. Ass. U.K. (1959) 38, 103–132
Printed in Great Britain

THE GROWTH STAGES OF THE LOPHOPHORE OF THE BRACHIOPODS *PLATIDIA DAVIDSONI* (EUDES DESLONGCHAMPS) AND *P. ANOMIOIDES* (PHILIPPI), WITH NOTES ON THE FEEDING MECHANISM

By D. Atkins, D.Sc.
From the Plymouth Laboratory

(Text-figs. 1–23)

The lophophore of *Platidia*, as illustrated in most memoirs and text-books, bears little resemblance to its appearance in life, no doubt because such illustrations and the accompanying descriptions were based on dried specimens. The lophophore of *Platidia* has been considered to be of a peculiar sigmoid type, differing from that of any other known lophophore. The dredging by R.V. 'Sarsia' of three species of the genus in recent years has afforded the opportunity of figuring the lophophore in its natural state, and the working out of its growth stages in *P. davidsoni* and *P. anomioides*: these were previously unknown for any species of *Platidia*. The third species, a new one, is described separately (Atkins, 1959).

Although *P. anomioides* (Philippi) is the type species of the genus, *P. davidsoni* (Eudes Deslongchamps) is considered first as it was the first to be dredged by R.V. 'Sarsia' and work on it was ready for publication when *P. anomioides* was obtained in May 1958.

All figures have been drawn with the aid of a camera lucida.

PLATIDIA DAVIDSONI (EUDES DESLONGCHAMPS)

A species of *Platidia* dredged by R.V. 'Sarsia' from off Penmarch and the north coast of Spain is evidently *P. davidsoni* (E. Deslongchamps) as it agrees with the description of that species by Eudes Deslongchamps (1855) in having 'small spinous asperities' on the outer surface of the ventral valve, although these are often confined to a submarginal zone, and in some individuals apparently worn and indistinguishable. Fischer (1872, 1873) noted of this species that 'elle porte quelquefois des aspérités assez nombreuses'. The brachial support agrees with the figures accompanying E. Deslongchamps's paper, but not with his description. There appears to be some confusion over its actual state. In his description of his new species, *Morrisia* (=*Platidia*) *davidsoni*, E. Deslongchamps (1855) described the 'apophysary system consisting of two lamellae originating at the base of the sockets, and united to a small elevated process or septum which arises from near the centre of the valve'. In the accompanying figure (pl. x, fig. 20, *c*, *d*) prongs at the apex of the septum are described as 'broken lamellae attached to the summit of the septum'. The prongs were evidently considered to be part of the descending and not of the ascending branches. It may be surmised that he found the branches as figured but assumed that they had been broken.

Fischer in 1872 and again in 1873 said of the brachial apparatus 'que nous n'avons pu obtenir entier, pas plus que M. Deslongchamps'.

Davidson in 1887 (p. 155) stated: 'Interior of the dorsal valve not completely known. Short cylindrical processes project into the interior of the shell from a little under each angle of the wide semicircular foramen, and from about the middle of the bottom of the valve arises a thickened pillar-shaped process or septum, curved toward the hinge, and forked at its extremity'. On the following page he mentioned that 'in *Platydia davidsoni* these principal stems, in the specimens examined by M. E. Deslongchamps, Dr Fischer, and myself, were either broken or absent; so that to ascertain their real condition more specimens will have to be examined hereafter'. Curiously enough Fischer & Oehlert in 1891 stated briefly 'appareil brachial comme dans le *P. anomioides*' and figured the brachial support as being exactly the same (pl. viii, fig. 15*b*, *c*) with the descending branches complete. It is difficult to understand their failure to mention that in previous descriptions by one of them (Fischer, 1872, 1873) and by Davidson (1887) the descending branches were described as either broken or absent.

The difference in the brachial support of the 'Sarsia' specimens and those of Fischer & Oehlert (1891) would not seem to be a size difference: their specimens had a length of 6 mm and width of 6·5 mm, whilst of my two largest *P. davidsoni*, one was 7·8 mm long and 7·2 mm wide and the other 7 mm long and 8 mm wide. In the 'Sarsia' specimens there is no doubt that the brachial support is as described and figured by Davidson in 1887. The absence of descending branches joined to the septum is not due to the fracture of extremely delicate branches in animals allowed to rot in sea water and then the remains blown out of the shell with a pipette. In a specimen 5·4 mm long and 6·9 mm wide which was sectioned, there is no indication of lateral outgrowths from the septal pillar other than the forked processes at the apex. In brachial valves with lophophore intact cleared in cedar wood oil or oil of winter green the same state of development was found.

It is not impossible that better development of the brachial support may occur in local varieties. However, as previous to Fischer & Oehlert's 1891 paper all authors had agreed that the descending branches in this species were either incomplete or broken, it would appear that their 'Travailleur' and 'Talisman' specimens need re-examination.

Platidia davidsoni was dredged in some numbers by R.V. 'Sarsia' in February and July 1956 and in June 1957 from a small area of yellow coral, *Dendrophyllia cornigera* Lamarck, off the Point of Penmarch at a depth of 45 to 48 fathoms. Two individuals, one 4·2 mm long and 6 mm wide and the other 2·7 mm long and 3·4 mm wide, were taken attached to a very large species of oyster off the north coast of Spain in position 43° 42′ N., 3° 58′ W. at a depth of 220 fathoms.

In the Penmarch area the *Platidia* were attached to the dead region of the growing coral and to the brachiopods *Terebratulina retusa* and *Megerlia truncata*, themselves fixed to the coral. They generally occurred on the lower brachial valves of the brachiopods in no definite position. Four *P. davidsoni* of shell length 0·8 to 2·7 mm were attached to the brachial valve of an adult *Megerlia*.

An occasional individual was found standing more or less erect on its hinge, with organisms encrusting both valves, but generally, when the animal is not feeding, the lower, brachial valve is closely applied to the substratum and when this has sharp irregularities the valve may be almost punctured. The brachial valve is thinner than the ventral, is generally approximately flat, but may be concave on coral. When situated where a slender side branch of coral arises the shell may show a well-marked artificial sulcus (see Fig. 11) where it extends on to the side branch.

The shell of *P. davidsoni* is frequently irregular in shape, owing to the irregularities of the coral and to the proximity of other growth. Occasionally when attached to the valve of another brachiopod the shell may be regular; an example was one near the anterior edge of the brachial valve of a *Terebratulina* and facing in the same direction. It was subcircular, 6 mm long and 7 mm wide, and regularly domed: half of its ventral valve was encrusted by a polyzoan. Irregularity in shell shape is accompanied by asymmetry in varying degree of the lophophore and sometimes by that of the gonad.

The mantle edge is produced into tiny lobes, and bears setae; in small individuals they reached a length of 0·6 mm.

Two spots of carmine pigment are present, probably in connexion with the preoesophageal ganglion. These pigment spots are difficult to distinguish, being masked by spicules: in some individuals they appear to be absent. They could not be found in *P. anomioides* and in a new species of *Platidia* (Atkins, 1959), both of which were taken in deep water. Carmine spots have also been found by me in certain other brachiopods.

From one *P. davidsoni*, 5·4 mm long, 6·9 mm wide, sectioned, with small gonad, it would seem that this species is hermaphrodite: the gonad was mostly male, with tailed sperm in the posterior region, but with a few small ova. From examination of entire specimens, however, it appears that ovaries and testes develop at different times, so that the animal appears dioecious. The ripe ovaries are dark orange and visible through the semi-transparent shell; the entire animal is orange tinted. The testes are of a less dark and more pinkish orange than the ovaries. *P. davidsoni* in the laboratory tanks retained their colour for about a month, but had lost much of it, especially from the filaments, after 2 months, possibly as a result of semi-starvation. Other brachiopods so far known to be hermaphrodite are *Argyrotheca* (see Senn, 1934) and *Pumilus antiquatus* (Atkins, 1958).

The gonads of *P. davidsoni* were large in February, June and July; specimens were not obtained at other times of the year.

GROWTH STAGES OF THE LOPHOPHORE AND ITS SUPPORT

During development the lophophore passes through the usual growth stages, trocholophous, schizolophous and zygolophous, culminating in a somewhat modified plectolophe.

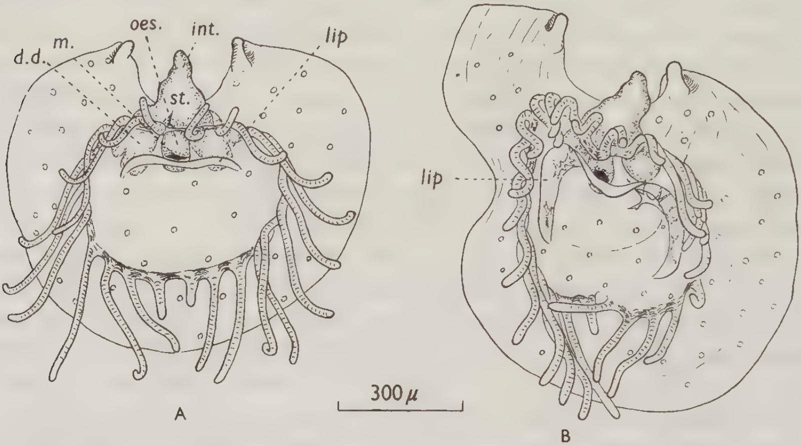


Fig. 1. *Platidia davidsoni*. Brachial valves of specimens of shell length 0·8 mm (A) and 1·0 mm (B) with trocholophes, to show the growth of the lip of the food groove (*lip*) in front of the mouth (*m.*): drawn living. In both individuals filaments are in single series. Spicules are present where indicated in the filaments and brachial membrane. Mantle caecae shown. In this and following figures mantle setae have been omitted. *d.d.*, digestive diverticulum; *int.*, intestine; *m.*, mouth; *oes.*, oesophagus; *st.*, stomach.

The trocholophous lophophore is of the broad-based terebratellacean type. The smallest individual obtained, of shell length 0·8 mm and width 0·9 mm, had the lophophore in this stage: the lophophoral ridge was a complete circle. Eleven filaments were present on the left and twelve on the right (Fig. 1 A). These were set low on the mantle, except for those behind the mouth which arose from the body. The lip of the food groove extended but a short distance on each side of the mouth. Spicules were present in the filaments and at their bases, but not elsewhere.

An unusually long-stalked, irregularly shaped individual of some 1 mm diameter, in the late trocholophous stage, had 14 pairs of filaments in single series: a few on the right were abnormally short. The lip had extended much further than in the smaller individual and two pairs of spicules were present in the brachial membrane (Fig. 1 B). These two specimens shown in Fig. 1 illustrate clearly the manner of growth of the lip of the food groove by lateral growth from a small preoral lobe, and not as an invagination of the rapid extension of the region between the latest formed filaments of the trocholophe (see Williams, 1956, for use of term) as described by Percival (1944) in *Terebratella inconspicua*.

At a shell length of 1·4 mm and width of 1·5 mm the lophophore was very slightly indented anteriorly: seventeen pairs of long filaments with ridged frontal surfaces were present in single series, and two pairs of minute buds. The lip of the food groove had now extended to the bases of the last long filament on each side. The number of spicules in the brachial membrane was two on one side and one on the other, so that some variation in the time of formation of these spicules evidently occurs.

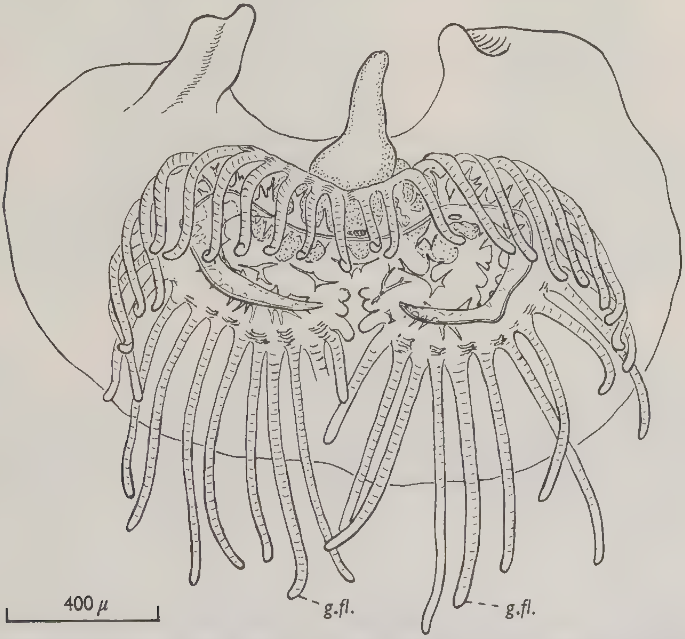


Fig. 2. *Platidia davidsoni*, of shell length 1·4 mm, width 1·7 mm. Brachial valve with early schizolophe, drawn living. Differentiation into a double series of filaments had begun, the first outer grooved filament on each side is indicated (*g.fl.*). The filaments behind the mouth are almost erect. The spicules supporting the lophophore have increased in size and numbers.

At a shell length of 1·4 mm and width of 1·7 mm the lophophore was early schizolophous with twenty-one pairs of long filaments and in addition three pairs of minute buds (Fig. 2). Differentiation of filaments had begun with three outer grooved filaments on the right and two on the left anteriorly. The spicules in the brachial membrane had increased in size and complexity.

The arrangement of the spicules at the base of the lophophore in an individual 1·8 mm long is shown in Fig. 3 A. At this size no septum could be distinguished in a valve with lophophore in position.

The trocholophe and early schizolophe are bell-shaped in the feeding position, the filaments are directed upwards and outwards (Fig. 3 B): it is only in the contracted state that they are directed inwards.

A more advanced schizolophous stage of shell length 2·0 mm and width 2·3 mm is shown in Fig. 4 A, and the appearance looking down into the gaping shell of a similar stage in Fig. 4 B. The brachial skeleton first appears at about this size in the form of a low septum, with tiny divergent lamellae, having the appearance in ventral view of a small transverse boss. That it represents small, almost horizontal, divergent lamellae on the apex of the septum is apparent from its appearance in transverse sections of an individual 2·3 mm long and 2·9 mm wide (Fig. 5). The septum was approximately 200 μ long and the conjoined bases of the lamellae 80 μ long antero-posteriorly, judging from the number of sections involved. The septum with the lamellae support the growing region of the lophophore and raise it above the valve floor, so that the brachial membrane slopes toward the mantle margins. At this stage the concavity formed by the upper surfaces of the lamellae is occupied by supporting substance similar to that present in the filaments. The two sections figured (Fig. 5) show the relation of the filaments to the mantle. Both small and great brachial canals are present at this stage.

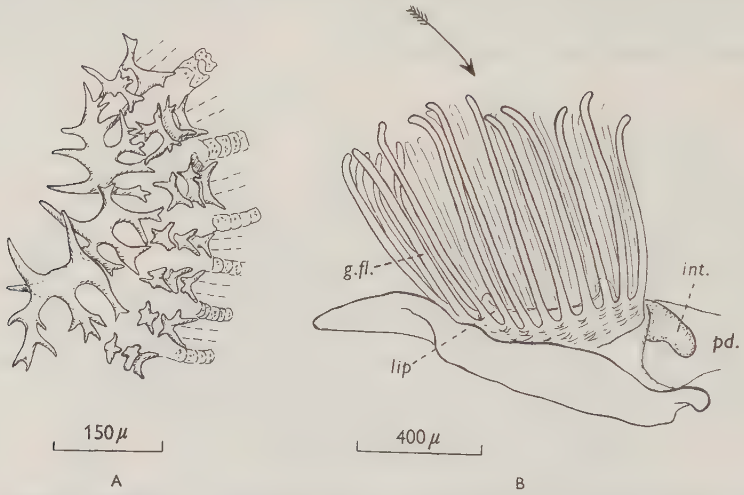


Fig. 3. *Platidia davidsoni*. A. Spicules of part of schizolophe of individual of shell length 1·8 mm, width 2·1 mm, viewed abfrontally, or dorsally. The inner filaments are indicated by broken lines. B. Side view of early schizolophe of an irregularly shaped individual of shell length 1·8 mm, drawn living. The arrow indicates the direction of the inhalant current. *g.fl.*, first outer, grooved filament; *int.*, intestine; *lip*, lip of the food grooves; *pd.*, pedicle.

The early zygolophous stage occurs between a shell length of 2·7 and 3·2 mm. At the smaller size the inner side of the lateral arms had not yet begun to be deflected, while at the larger size this inclination had begun (Fig. 6).

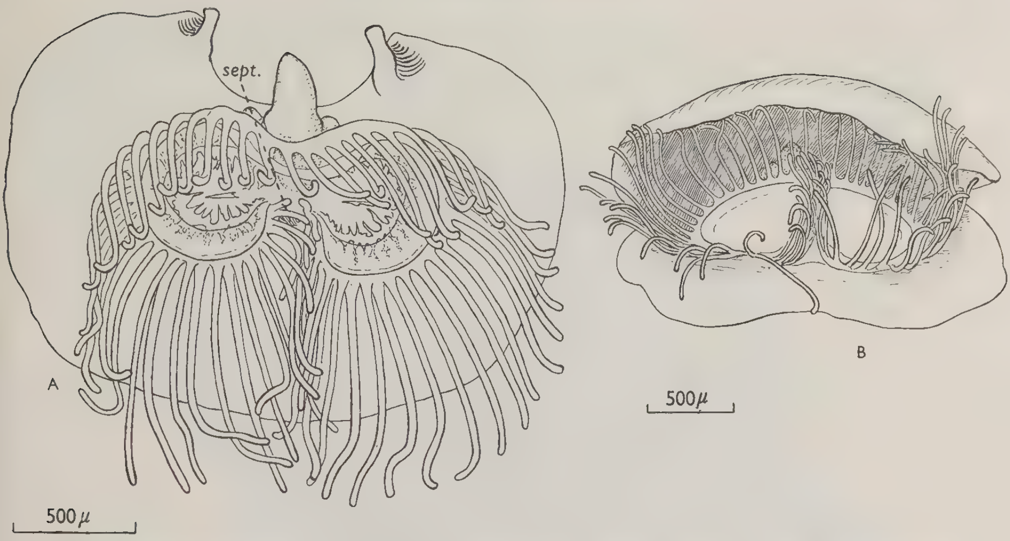


Fig. 4. *Platidia davidsoni*. A. Specimen of shell length 2·0 mm, width 2·3 mm: brachial valve with schizolophe, drawn living. The filaments behind the mouth are almost erect; a septum is present. Only the larger spicules are shown. B. Specimen of shell length 2·2 mm, width 2·3 mm: view looking down into the shell, drawn living, but narcotized.

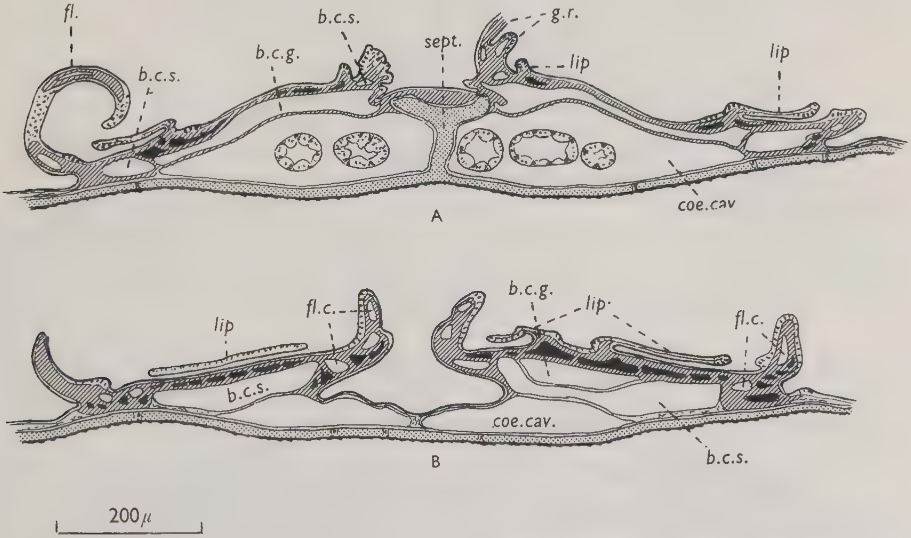


Fig. 5. *Platidia davidsoni*, of shell length 2·3 mm, width 2·9 mm. Transverse sections through the brachial valve with schizolophe: (A) through the septum (*sept.*) with its divergent lamellae, and the twin growing regions of the lophophore; (B) through the anterior region of the lophophore, slightly oblique. On the right through the anterior recurvature of the small brachial canal; on the left slightly more anterior, so that the great brachial canal is absent. *b.c.g.*, great and *b.c.s.*, small brachial canals; *coe.cav.* coelomic cavity; *fl.*, filament; *fl.c.*, filamentar canal; *g.r.*, growing region of right side; *lip*, lip of food groove. Spaces formerly occupied by spicules shown black; supporting substance shown hatched.

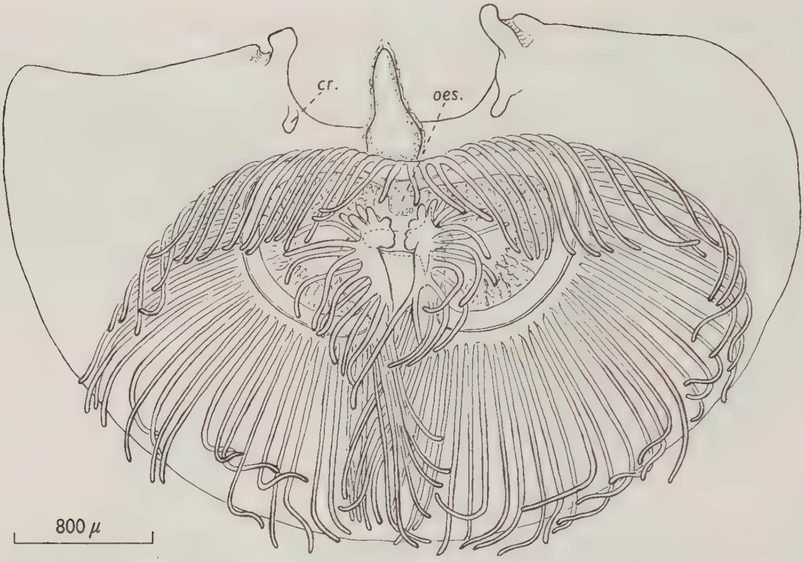


Fig. 6. *Platidia davidsoni*, of shell length 3·2 mm, width 4·6 mm. Brachial valve with zygolophe; the inner sides of the lateral arms have begun to turn outwards. Drawn living: brachial support, indicated by broken lines, added after clearing in cedar wood oil. *cr.*, crura; *oes.*, oesophagus.

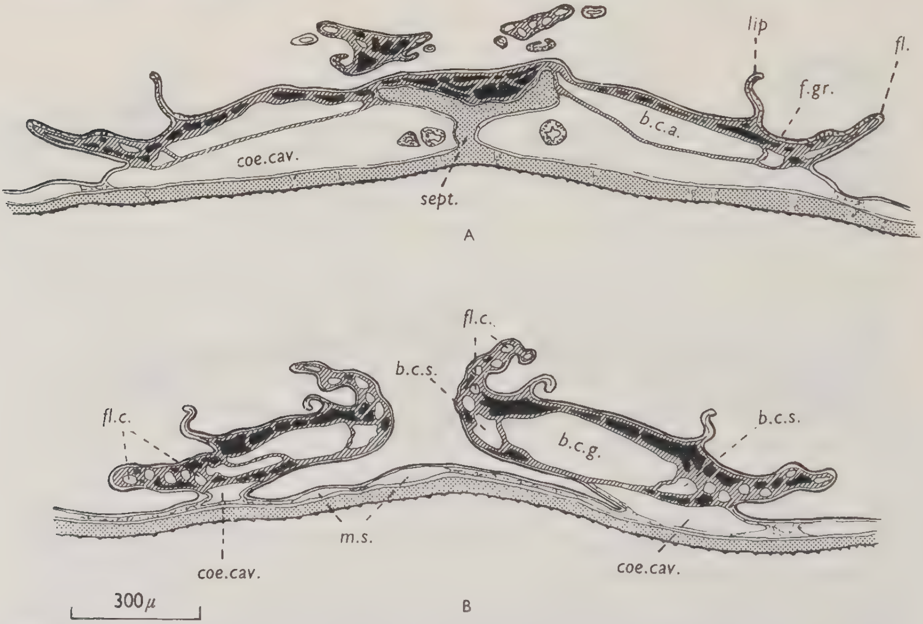


Fig. 7. *Platidia davidsoni*, of shell length 3·1 mm, width 4·1 mm. Transverse sections through the brachial valve and early zygolophe of a slightly later stage than shown in Fig. 6: (A) through the septum (*sept.*) and its divergent lamellae, (B) near the anterior end of the lophophore. The two lateral arms face ventrally, but slope slightly toward the mantle margins. The lateral arms are beginning to separate from the mantle anteriorly. *f.gr.*, food groove; *m.s.*, mantle sinus; other lettering as in Fig. 5.

A specimen 3·1 mm long and 4·1 mm wide was sectioned: the sections illustrated (Fig. 7) show the almost horizontal lamellae at the crest of the septum. The lophophore is being gradually raised above the valve floor, not only by the septum and its lamellae, but also by the lateral extension and increase in size of the coelomic cavity (Fig. 7A). More anterior to the section figured (Fig. 7B) the lophophore is free from the valve floor for a very short distance. At this size crura have begun to develop (Fig. 6). The brachial support of an individual of greater length (3·5 mm), but lesser width (3·2 mm) is shown in Fig. 12A, B: the septal pillar is continued posteriorly as a low ridge almost to the edge of the foramen. The posterior prolongation of the septal pillar is variable in development, for in the larger shell to be described it extended only a short distance (Fig. 8).

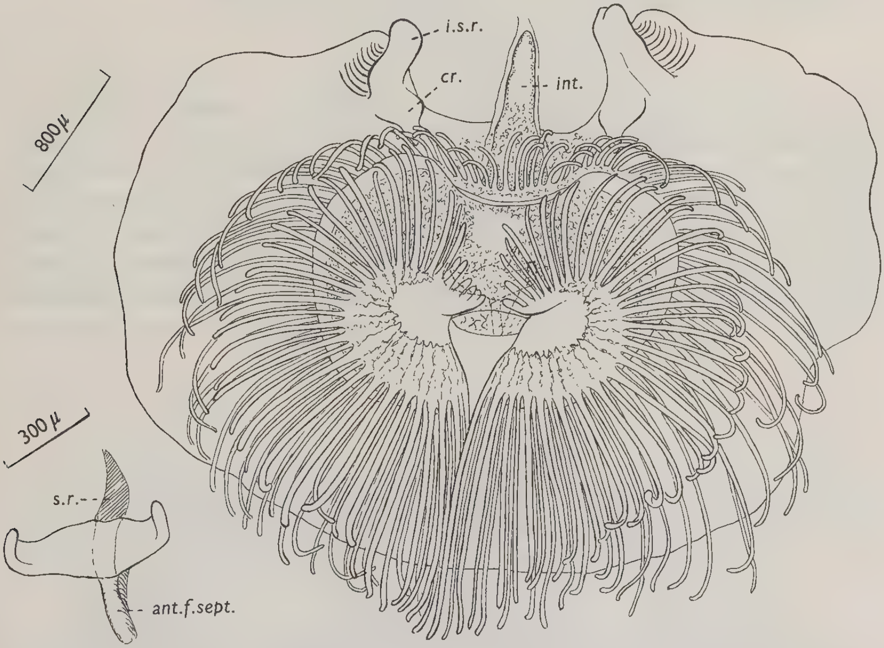


Fig. 8. *Platidia davidsoni*, of shell length 3·7 mm, width 4·4 mm. Brachial valve with late zygolophe, drawn living. Brachial support, indicated by dotted lines, added after clearing in cedar wood oil. Inset septum and divergent lamellae enlarged. *ant.f.sept.*, anterior face of septum; *cr.*, crura; *int.*, intestine; *i.s.r.*, inner socket ridge; *s.r.*, low septal ridge.

The late zygolophous stage occurred in an individual 3·7 mm long and 4·4 mm wide (Fig. 8). It appears to be of the normal type, except that the reflected portion of the lateral arms does not reach as far posteriorly as in most genera with plectolophes in the adult. The septum still bears what appear to be broad divergent lamellae, and these are produced on their outer ends into small ventrally curved processes (Fig. 8, inset). Possibly the latter are rudiments of a transverse band, or it may be that the lamellae are undergoing transformation into the curved prongs or short ascending branches of the adult. The divergent lamellae resemble those in immature *Kraussina rubra* (Elliott, 1949) and in the adult *Pumilus antiquatus* (Atkins, 1958), but are set at a much wider angle to each other, being almost horizontal, and are borne on a high septal pillar. Crura are still short at this size.

It may be noted that the low posterior end of the septum sometimes passes on the right and sometimes on the left of the oesophagus.

THE ADULT LOPHOPHORE

Eudes Deslongchamps (1855), the discoverer of *P. davidsoni*, having dried specimens only, neither described in any detail nor figured the lophophore. Later writers have described the lophophore of *Platidia* as being of a peculiar sigmoid type. Fischer (1872, pl. vi, fig. 5) figured that of *P. davidsoni* with the filaments extended, although not fully. The darkly shaded circular area on the oral disc evidently represents the part not covered by spicules. Figures by Fischer & Oehlert (1891, pl. viii) of both *P. anomioides* and *P. davidsoni* show the lophophore much contracted, and were probably drawn from dried specimens.

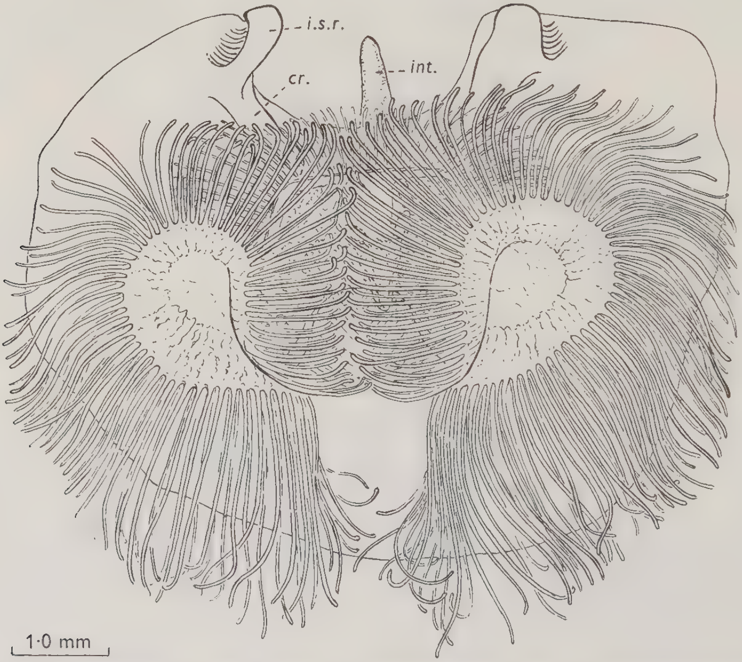


Fig. 9. *Platidia davidsoni*, of shell length 6·2 mm, width 7·0 mm. Brachial valve with fully adult plectolophe, drawn living. The spiral arm consists of one short coil. The brachial support added after clearing in cedar wood oil, but not clearly indicated. *cr.*, crura; *int.*, intestine; *i.s.r.*, inner socket ridge.

The adult lophophore of *P. davidsoni* as seen in the living animal is a somewhat modified plectolophe with a short spiral arm of little more than one coil (Fig. 9). This arm is held well away from the brachial valve by the septal pillar and the body (Figs. 10, 11). In the normally feeding animal the appearance is of a broad plectolophous lophophore.

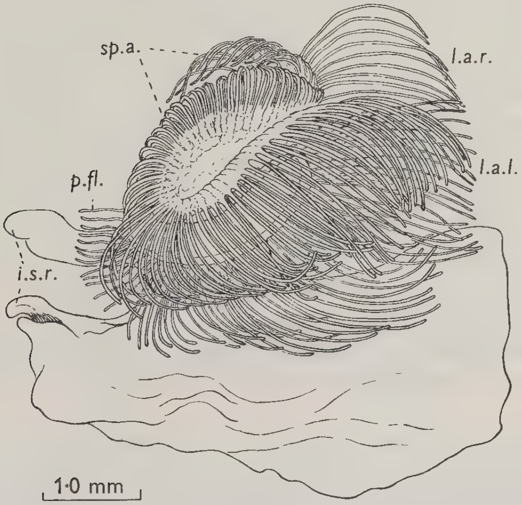


Fig. 10. *Platidia davidsoni*, of shell length 5·5 mm, width 7·0 mm; shell irregular. Side view of plectolophe, drawn after narcotizing, fixing and clearing in cedar wood oil. *i.s.r.*, inner socket ridge; *l.a.l.*, left and *l.a.r.*, right lateral arm; *p.fl.*, filaments behind mouth; *sp.a.*, spiral arm.

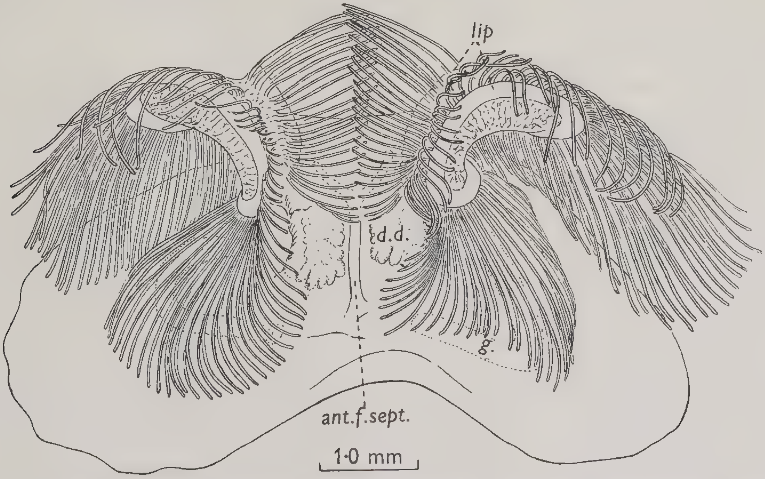


Fig. 11. *Platidia davidsoni*. Anterior view of brachial valve and plectolophe, the ventral side only of the spiral arm shown. Same individual as in Fig. 10, but drawn living. Shell irregular with artificial sulcus caused by the surface of attachment. *ant.f.sept.*, anterior face of septum; *d.d.*, digestive diverticulum; *g.*, gonad; *lip*, lip of food groove.

In the schizolophous stage, as Morse (1871, p. 420–1) noted in *Terebratulina septentrionalis*, and as probably in this stage in all brachiopods, the brachial membrane, or floor of the lateral arms, faces ventrally, and is almost parallel with the floor of the brachial valve, although raised to a varying degree medianly. As the zygolophous stage approaches, the lateral arms become deflected, the floor facing outwards and lying almost at right angles to the plane of the brachial valve. In most plectolophes this position is more or less retained, but in *Platidia davidsoni* the inclination continues so that the floor partly faces dorsally and the line of the bases of the ventral row of filaments lies considerably nearer the lateral edges of the shell than do the bases of the dorsal row. In an animal fixed without narcotizing the lateral arms face almost entirely dorsally (Fig. 13 E) and this is probably the position when the shell is closed. The floor of each lateral arm is short and broad and its posterior edge is incurved. The spiral arm is short and broad, and lies largely ventral to the lateral arms. The membrane connecting the two sides is deeply embayed anteriorly, as in *Megerlia truncata*. The twin growing regions of the lophophore at the apex of the spiral lie dorsally against the floor of this arm, which is an anterior continuation of the oral disc (see Richards, 1952, for use of term).

The adult brachial skeleton is slightly developed (Fig. 12 C, D). The crura are short, not reaching the posterior edge of the lophophore (Figs. 9, 10). The broad, nearly horizontal, lamellae at the apex of the septal pillar of the young stages of the lophophore have changed into the prongs or short ascending branches of the adult and curve around the sides of the oesophagus. They support the median region of the oral disc. In individuals of length 4 mm and over, the septal pillar is continued posteriorly as a low ridge to the edge of the pedicle foramen.

The lophophore is heavily spiculated, compensating for the slight development of the brachial skeleton. The oral disc is covered with interlocking plate-like spicules with some windows, except for a small area over the oesophagus, extending to the apex of the septum. The floor, or brachial membrane, of the lateral arms is also covered with plate-like spicules, except for a small median area toward which spine-like branches from the spicules project. On the abfrontal surface of the lateral arms similar spicules occur in a deep band at the bases of the filaments, but leaving a large median naked area (Fig. 9).

Small and large brachial canals are present in the lophophore: the former give off branches to the filaments (Fig. 13). The large brachial canal of each lateral arm is confluent for much of its length with that of the neighbouring part of the spiral arm (see Fig. 13). In a specimen 5·4 mm long and 6·9 mm wide, sectioned, the extension of the coelomic cavity into each lateral arm—known as the brachial pouch—was exceedingly short (Fig. 13 C).

A 'blood' vessel is present in the usual position within the small brachial canal and a branch is given off to each filament.

The development of muscle fibres in the walls of the canals is slight.

The principal (*n.p.*) and external (*n.ext.*) nerves of the lophophores are clearly discernible, while the secondary nerve (*n.sec.*) is difficult to distinguish. At the magnification of the section drawn (Fig. 13F) no more than the position of the nerves can be indicated.

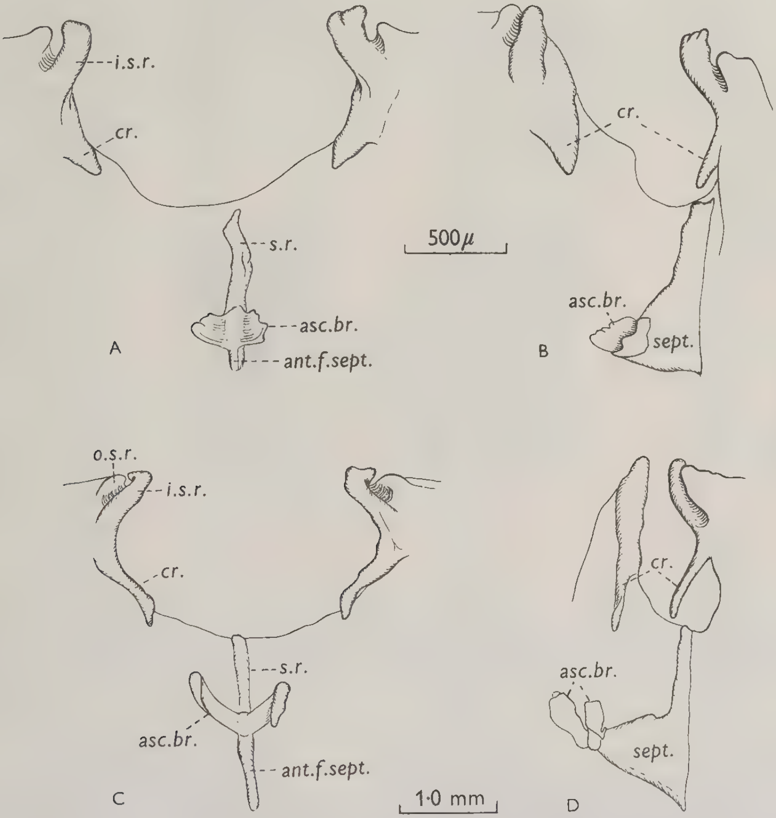


Fig. 12. *Platidia davidsoni.* Brachial support of: A and B, individual of shell length 3·5 mm, width 3·2 mm; C and D, individual of shell length 6·6 mm, width 8·4 mm. A and C ventra views; B and D lateral views. *ant.f.sept.*, anterior face of septal pillar; *asc.br.*, ascending branch; *cr.*, crura; *i.s.r.*, inner, and *o.s.r.*, outer socket ridges; *s.r.*, septal ridge; *sept.*, septum.

The distribution of the mucous gland cells on the lophophore is similar to that in other plectolophes. The abfrontal band below the filaments—in the exhalant chamber—is narrow, but the cells are deep. A narrow band of deep gland cells is present on the brachial membrane at the base of the lip of the food groove (Fig. 13F). Scattered mucous cells occur in the walls, including the lip, of the food groove.

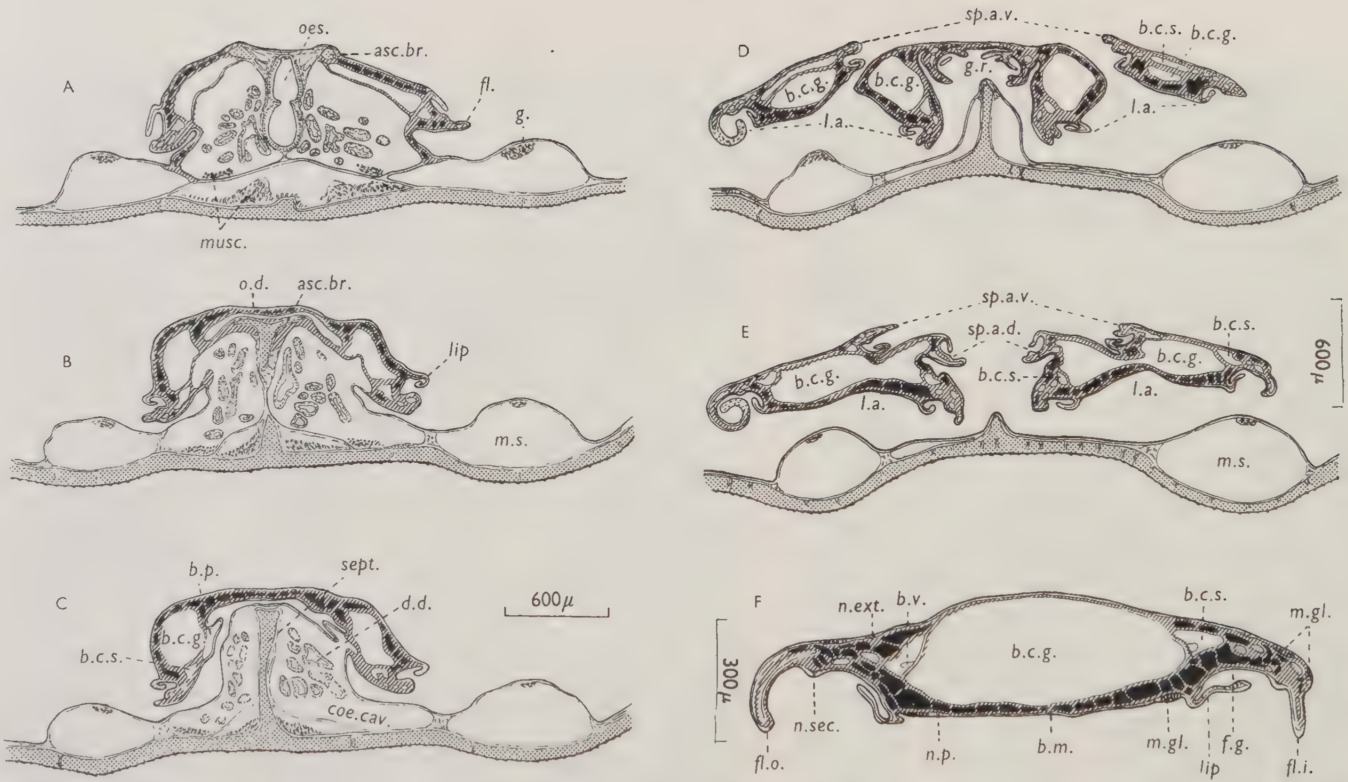

A
oes.
asc.br.
fl.
g.
musc.
B
o.d.
asc.br.
lip
m.s.
C
b.p.
sept.
d.d.
600μ
b.c.g.
b.c.s.
coe.cav.
D
sp.a.v.
b.c.s.
b.c.g.
b.c.g.
b.c.g.
g.r.
l.a.
l.a.
E
sp.a.v.
sp.a.d.
b.c.s.
b.c.g.
b.c.g.
b.c.s.
l.a.
l.a.
600μ
m.s.
F
n.ext.
b.v.
b.c.s.
m.gl.
b.c.g.
300μ
n.sec.
fl.o.
n.p.
b.m.
m.gl.
lip
f.g.
fl.i.

Fig. 13. *Platidia davidsoni*, of shell length 5·4 mm, width 6·9 mm. Transverse sections of brachial valve, fixed without being narcotized and therefore with plectolophe contracted: A, through lophophore, oesophagus (*oes.*)—showing the valve-like elevation of the wall—and the ascending branches; B, through the oral disc (*o.d.*) and junction of ascending branches with the septum; C, anterior to the ascending branches—on the right the lophophore is still attached to the body, on the left a small brachial pouch (*b.p.*) is present; D, through the growing regions (*g.r.*) of the lophophore at the apex of the spiral arm; E, the floor of the lateral arms faces dorsally; F, transverse section of lateral arm in position in closed shell, with the brachial membrane or floor facing the brachial or dorsal valve. *asc.br.*, ascending branches; *b.c.g.*, great, and *b.c.s.*, small brachial canals; *b.m.*, brachial membrane; *b.v.*, 'blood' vessel; *coe.cav.*, coelomic cavity; *d.d.*, digestive diverticula; *fl.*, filament; *fl.i.*, inner and *fl.o.*, outer filaments and bases; *f.g.*, food groove; *g.*, gonad; *l.a.*, lateral arm; *lip*, lip of food groove; *m.gl.*, mucous gland cells; *m.s.*, mantle sinus; *musc.*, muscle; *n.ext.*, external; *n.p.*, principal; and *n.sec.*, secondary nerves; *sept.*, septum; *sp.a.d.*, dorsal and *sp.a.v.*, ventral side of spiral arm. Spaces formerly occupied by spicules shown black: supporting substances shown hatched.

The filaments are arranged in a double alternating series of inner filaments, with ridged frontal surfaces, and outer filaments with grooved frontal surfaces, but behind the mouth some sixteen or seventeen pairs are in single series and of the ridged type. Spicules are present in both types of filament. They are broad transverse bands, granulated and somewhat irregular in outline, present abfrontally within the tube of supporting substance and curving around the sides of the filaments (Figs. 14, 15). The supporting substance in brachiopods was described as semi-cartilagenous by Hancock (1858), but according to Prenant (1928) working on *Terebratulina retusa* (=*caput-serpentis*) it is more nearly like collagen: he preferred to call it simply 'substance de soutien'.

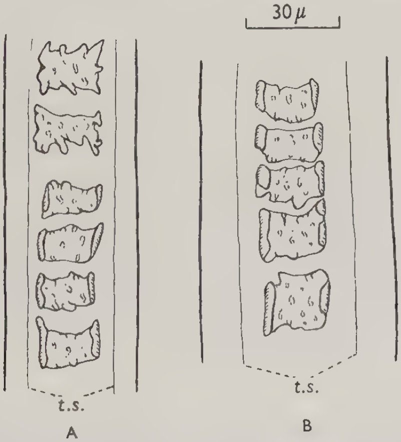


Fig. 14. *Platidia davidsoni*. Spicules in two adjacent filaments at the same distance from the food groove, viewed from the frontal surface. A, an inner filament, with ridged frontal surface; B, an outer filament, with grooved frontal surface. *t.s.*, tube of supporting substance.

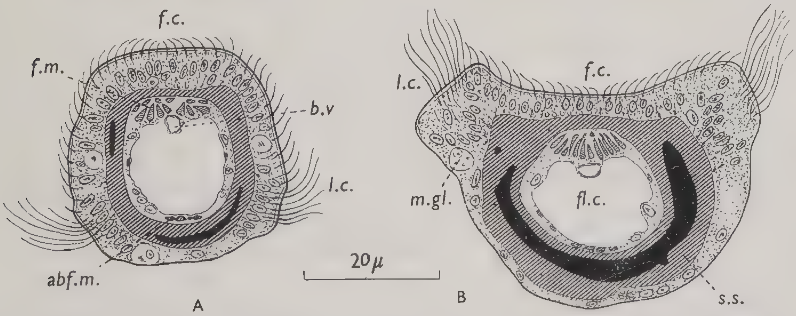


Fig. 15. *Platidia davidsoni*. Transverse sections of A, inner filament, with ridged frontal surface; B, outer filament, with grooved frontal surface. *abf.m.*, abfrontal muscles; *b.v.*, 'blood' vessel; *f.c.*, frontal cilia; *fl.c.*, filamentar canal; *f.m.*, frontal group of muscles; *l.c.*, lateral cilia; *m.gl.*, mucous gland cell; *s.s.*, supporting substance. Spaces formerly occupied by spicules shown black. Frontal cilia not shown at their full length, as they beat along the filament.

Longitudinal muscle fibres are present in the walls of the filamentar canal, especially well developed in a striated frontal group and to a lesser extent abfrontally (Fig. 15); the latter appear to be smooth.

The structure of the lophophore of *Platidia* is peculiarly adapted to the shallow mantle cavity of the closed shell. In separating and closing the valves of a detached living animal it is possible to see something of the normal movements of the lophophore. As the valves are closed the anterior ends of the side arms approach each other in the mid-line and the whole lophophore appears to be flattened by the approximation of the valves. As the valves are separated the lophophore is raised, although under these conditions the filaments remain tightly coiled. It would seem that it is so constructed that it can be flattened by the approximation of the valves, and fitted into the small dorso-ventral space available: the largely spicular support of the lophophore, and the floor of the lateral arms being approximately parallel with the valve floor making this possible. When the valves are gaping widely, as naturally when feeding, there is ample space for the spiral arm to expand.

When the valves are closed the prongs at the apex of the septal pillar almost touch the floor of the ventral valve, as can be observed through the foramen of an empty shell. This close proximity of the median region of the brachial support to the ventral valve in the closed shell seems not unusual in brachiopods. In young *Macandrevia cranium* the apex of the hood almost touches the pedicle valve when the shell is closed.

PLATIDIA ANOMIOIDES (PHILIPPI)

On 13 May 1958, over sixty *P. anomioides* from 1·1 to 4·6 mm long were dredged by R.V. 'Sarsia' somewhat to the south-east of La Chapelle Bank in position 47° 25′ N., 6° 30′ W. in 400–405 fm. They were attached to the dead region of probably growing coral, *Lophelia prolifera* (L.), in company with the more plentiful *Pantellaria echinata* (Fischer & Oehlert), and some few *Megathyris* sp.[1] Most were of the regular, transversely oval, shape illustrated by Philippi (1844, pl. 18, fig. 9) and Fischer & Oehlert (1891, pl. 8, fig. 14), no doubt because the surface of the coral was smooth and fairly free from other organisms, but some were subrectangular and a few irregular in shape. One of the largest individuals, an irregular one, had a shell length of 4·4 mm and width of 5·0 mm. This is somewhat larger than the size given for this species by Davidson (1887, p. 153) of diameter 2 lines, that is just over 4 mm, but considerably less than the length of 6 mm and width of 7 mm given by Fischer & Oehlert (1891, p. 95). Massy (1925) records a maximum size of *ca.* 5 × 6 mm of specimens taken on *Lophohelia* (= *Lophelia*) off the south-west of Ireland. The young are occasionally rather longer than broad, but with

[1] Bivalves on the coral were few.

growth tend to become as wide or wider than long. The mantle margin is furnished with fairly short setae, as in *P. davidsoni.*

The numbers of *P. anomioides* obtained has allowed of the working out of the growth stages of the lophophore, and in particular of the brachial support, which is considerably further developed than in *P. davidsoni.*

THE GROWTH STAGES OF THE LOPHOPHORE AND ITS SUPPORT

The lophophore of *P. anomioides* unfortunately did not expand well after separation of the valves; stovaine failed to give good results and magnesium chloride did not always act well.

The lophophore passes through the growth stages described for *P. davidsoni.* The filaments appear to be generally longer than in that species—compare, for instance, Figs. 3B and 16 and Figs. 9 and 21—and a greater number are in single series behind the mouth, some 25 or 26 pairs as compared with 16 or 17 pairs in *P. davidsoni.*

The lophophore is trocholophous (Fig. 16) up to a shell length of about 1·6 mm: it then becomes slightly indented in the mid-line anteriorly. A minute triangular septum and short crura are present. The individual figured (Fig. 17), of shell length 1·8 mm and width 1·9 mm, had the lophophore poorly expanded, but clearly shows the relation of the septum to the anterior median invagination. All the filaments were in a single row and were spiculated: spicules were also present in a wide band at their bases. Fig. 18 shows the characteristic arrangement of a large spicule on each side of the septum, the two converging anteriorly. At a diameter of some 2 mm the crura were longer than shown in Fig. 17, and the triangular septum somewhat higher, but as yet not bifurcated (Fig. 23A). Minute projections were present at its apex at a shell length of 2·3 mm and width 2·5 mm (Fig. 20A).

The late schizolophous stage was found at a diameter of 2·5 mm: the inner side of the side arms or lobes had begun to turn outwards, this stage corresponding to that figured for *P. davidsoni* at a length of 3·2 mm (see Fig. 6). The crura were short and reached to just behind the lophophore; crural processes were absent. The apex of the septum was forked; anterior ends of the descending branches if present must have been short as they were not distinguishable in the uncleared animal. It would appear that in this individual the lophophore was somewhat retarded in development as the zygolophous stage occurred in three individuals of length 2·4 mm, width 2·8 mm (Fig. 19); length 2·6 mm, width 2·9 mm; and length 2·6 mm, width 2·7 mm. In the first two of these the twin-growing regions of the lophophore were close together, in the last they were separated. In all three crural processes were present, although short: the posterior ends of the descending branches were either short or not yet formed; the anterior ends were present and longest in the third specimen. The ascending branches at the apex of the septum were best developed in the second specimen. At a length and width of of 3·3 mm

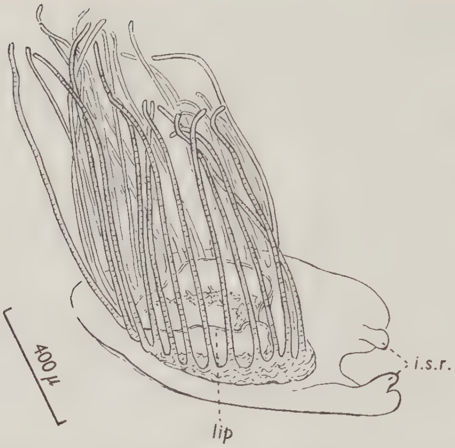


Fig. 16. *Platidia anomioides*, shell length 1·2 mm, width 1·3 mm. Side view of brachial valve with trocholophe, drawn narcotized. Spicules indicated, except in filaments of the far side. *i.s.r.*, inner socket ridge; *lip*, lip of food groove.

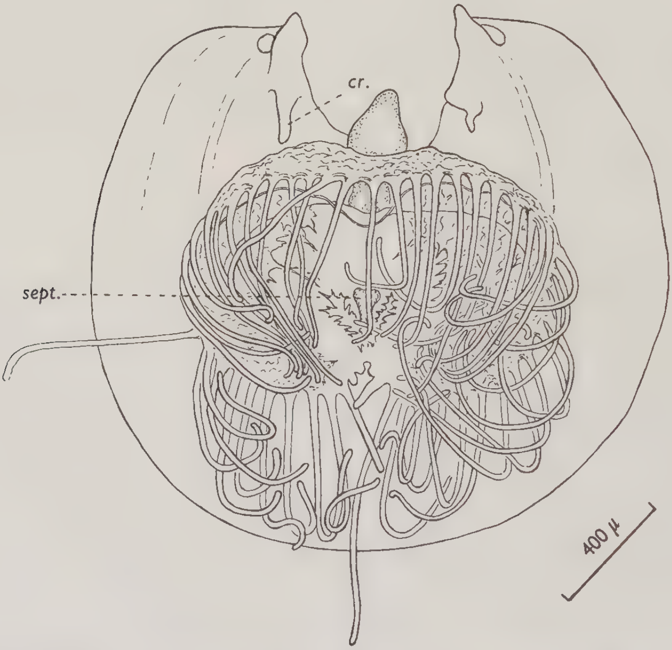


Fig. 17. *Platidia anomioides*, shell length 1·8 mm, width 1·9 mm. Brachial valve with early schizolophe, drawn unsuccessfully narcotized. It shows the relation of the septum (*sept.*) to the anterior median invagination of the lophophore. *cr.*, crura, *sept.*, septum.

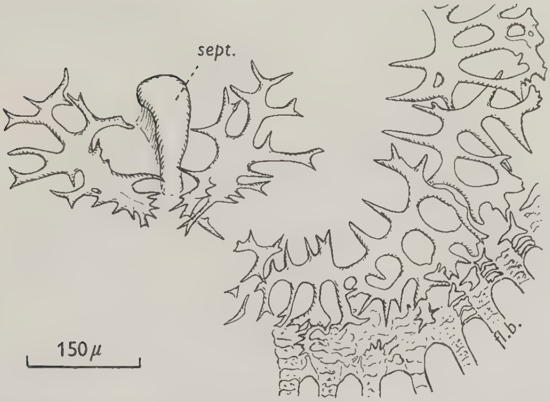


Fig. 18. *Platidia anomioides*, of length 1·9 mm, width 2·2 mm. Spicules of part of schizolophe viewed ventrally. The large spicules, one on each side of the septum (*sept.*) are to be noted. *fl.b.*, bases of filaments in single series at this stage.

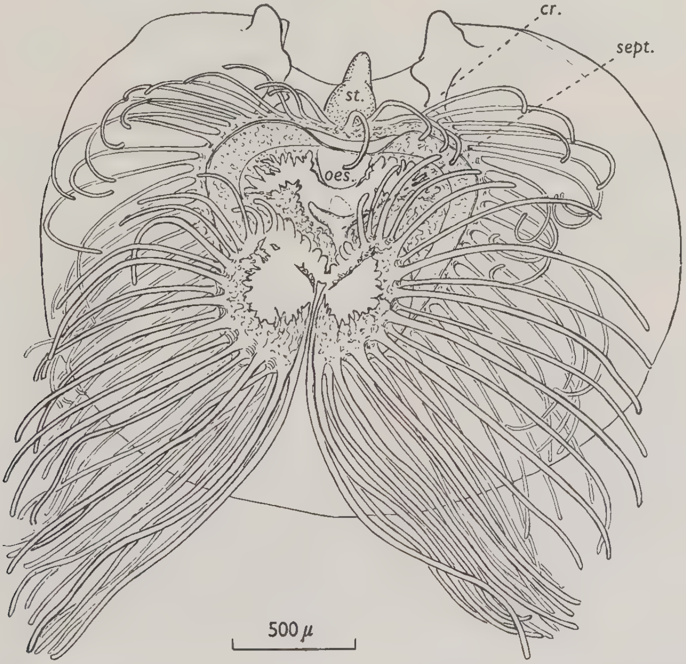


Fig. 19. *Platidia anomioides*, of length 2·4 mm, width 2·8 mm. Brachial valve with zygolophe, the twin growing regions close together: drawn narcotized. *cr.*, crura; *oes.*, oesophagus; *sept.*, septum, with ascending branches; *st.*, stomach.

the lophophore was late zygolophous or very early plectolophous with the growing regions well separated: this stage corresponds to that figured for *P. davidsoni* (Fig. 8) at 3·7 mm long and 4·4 mm wide. The animal was a female with large ova.

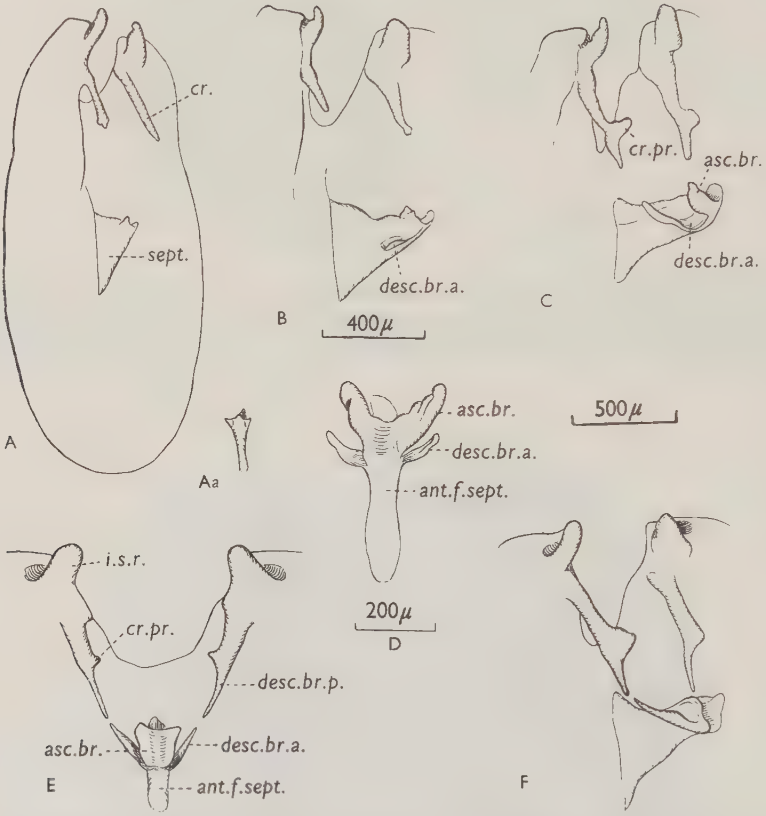


Fig. 20. *Platidia anomioides.* To show the development of the brachial support: lateral views unless otherwise stated. A, shell length 2·3, width 2·5 mm; A*a*, ventral view of septum; B, shell length 2·8 mm, width 2·9 mm; C, shell length 2·4 mm, width 2·8 mm; D, shell length 2·9 mm, width 3·5 mm; ventral view of septum with perhaps unusually large ascending branches; E and F, shell length 2·9 mm, width 3·1 mm: E, ventral view of septum. *ant.f.sept.*, anterior face of septum; *asc.br.*, ascending branches; *cr.*, crura; *cr.pr.*, crural process, *desc.br.a.*, anterior and *desc.br.p.*, posterior ends of descending branches; *i.s.r.*, inner socket ridge; *sept.*, septum. All except B and D to scale on right.

As fine details of the brachial support are not easily discernible in valves in which the flesh is present, they will be described from those in which it had been removed. The anterior ends of the descending branches had begun to grow from the septum in an individual of shell length 2·8 mm and width 2·9 mm (Fig. 20B). The crura were short and crural processes absent. In one

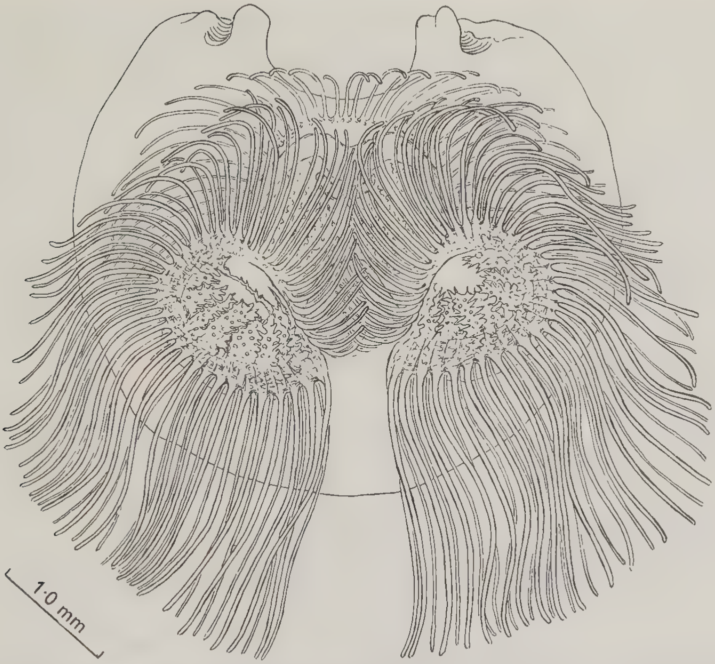


Fig. 21. *Platidia anomioides*, of shell length 4·6 mm, width 4·4 mm. Brachial valve with plectolophe, drawn living and narcotized.

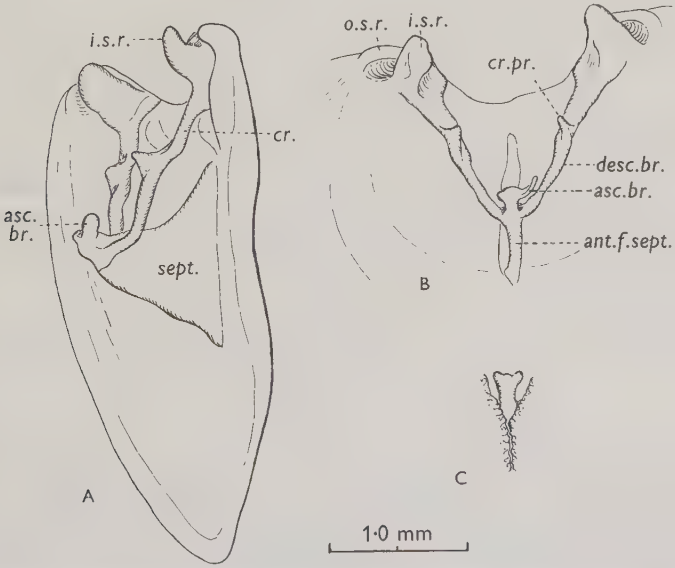

i.s.r.
cr.
asc. br.
sept.
A
o.s.r.
i.s.r.
cr.pr.
desc.br.
asc.br.
ant.f.sept.
B
C
1·0 mm

Fig. 22. *Platidia anomioides*, of shell length and width approximately 4 mm. A, side view of brachial valve; B, ventral view of brachial support, with the ascending branches unequally developed; C, ventral view of the septum of another individual of about the same size. *o.s.r.*, outer socket ridge; other lettering as in Fig. 20.

of smaller size, length 2·4 mm and width 2·8 mm, the brachial support was more advanced in development. The anterior ends of the descending branches were longer and had moved up the septum to near the origin of the ascending branches: the posterior ends had begun to grow from the crura, on which short crural processes were present (Fig. 20C). The ascending branches at the apex of the septum are variably developed in *P. anomioides*, but are generally very short even in the adult. Fig. 20D is an anterior view of a septum with rather well-developed ascending branches of an individual of length 2·9 mm and width 3·5 mm.

The anterior and posterior ends of the descending branches had almost met in a specimen of length 2·9 mm and width 3·1 mm. The ascending branches were extremely short, appearing as two short lamellae on the apex of the septum (Fig. 20E, F).

The adult lophophore (Fig. 21) is like that of *P. davidsoni*. The floor of the lateral arms, however, is perhaps even more fully dorsally facing: the lateral arms have longer filaments anteriorly and their abfrontal surfaces are more entirely covered by spicules. The spiral arm is small, of one coil only. The dorsal filaments of the lateral arms extend but a short distance beyond the edge of the brachial valve, except anteriorly where they lengthen as they become continuous with the long ventral filaments. The lophophore when expanded slants upwards at an angle of some 45° with the surface of the dorsal valve, so that it is raised high above the valve anteriorly. This anterior upward tilting of the lophophore is found also in *P. davidsoni* (see Fig. 10). The oral disc is covered by spicules except for a small area over the oesophagus and septal pillar. In the mid-line a well-marked separation is evident of the spicules of the left and right sides; it runs from the septum to the anterior edge of the oral disc (Atkins, 1959, fig. 7B). The abfrontal surface of the lateral arms is also very fully covered by spicules, only a small area remaining free from them in specimens of about 4 mm and more in diameter. The brachial skeleton of *P. anomioides* of rather more than 4 mm is shown in Fig. 22A, B. The ascending branches are unevenly developed, that of one side being perhaps unusually long. The apex of the septum of another specimen of about the same size is shown in Fig. 22C; the ascending branches were short.

PREVIOUS ACCOUNTS OF THE BRACHIAL SUPPORT OF *PLATIDIA ANOMIOIDES* (PHILIPPI)

The first account to be published of *Platidia anomioides* was that by Philippi in 1844, who called it *Orthis anomioides*, adopting a manuscript name of Scacchi. The brachial support and lophophore were described as follows: 'Sceleton internum septum triangulare, ut in praecedente ostendit, sed non simplex est; margo aperturam respiciens enim canaliculatus et apex incrassatus utrinque bicarinatus, inter carinas foveolatus est. Brachia circulos perfectos describunt, cirrosque externe gerunt'. No mention was made of descending branches. The length of the shell was given as $1\frac{3}{4}'''$, and the width as $2'''$.

In January 1852 Costa gave the name *Platidia* to the genus of which *P. anomioides* was the type and only species. Davidson unaware of this, in May 1852*a* (p. 371) gave the name *Morrisia* to the genus and described 'the apophysary system consisting of two branches, originating at the base of the dental sockets, and united to a small elevated process arising from the centre of the valve'. As the type of the genus he gave *M. seminulum*. One of the accompanying figures appears to show two minute horns at the apex of the septum, although these are not mentioned in the text. The other figure shows the lophophore 'with two subspiral or *sigmoid* arms' and the filaments quite well extended: a spiral arm, however, is not shown. It was apparently drawn from a dried specimen (see Davidson, 1887, p. 153). The above description of the lophophore and its support was repeated under the name *Morrisia anomioides* by Davidson (1852*c*) in the same year in the *Proc. Zool. Soc.*

In 1887 (p. 153) Davidson's description was as follows: 'in the interior of the dorsal valve the loop is not reflected. The converging principal lamellae are found attached first to the hinge-plate, and then to a small pillar-shaped median vertical septum'. The accompanying figure (1887, pl. 21, fig. 18) shows no horns on the septum.

Fischer & Oehlert (1891, p. 94) described and figured the brachial support as more fully developed than in any previous account. They said: 'De la base de ces apophyses fovéales s'élèvant presque verticalement deux cruras longs et grêles, limités par deux fines pointes crurales, au delà desquelles les branches descendantes, minces et ténues, vont en convergeant rejoindre un pilier septal très élevé, auquel elles se soudent vers les trois quarts de sa hauteur. Le pilier est constitué par une lamelle triangulaire remontant jusqu'au bord de l'échancrure; cette lamelle, peu épaisse, est arquée vers l'arrière et légèrement renflée au sommet, qui se termine par deux pointes divergentes courtes et assez massives, supportant la membrane brachiale médiane'.

It is most probable from the appearance of the shell and pedicle opening that Philippi's *P. anomioides* is the same shell as now goes under that name, although his description of the brachial support, without descending branches, would apply to *P. davidsoni*. Philippi's specimen of *P. anomioides*, about 3·5 mm long and about 4 mm wide, is of a size to have the descending branches complete if they had not been broken.

THE IDENTITY OF *TEREBRATULA SEMINULUM* PHILIPPI 1836

Philippi in 1836 published a short account with figures (pl. vi, fig. 15) of a small shell which he called *Terebratula seminulum*. His description is as follows: 'Testa circa $1'''$ longa, totidem lata, mox exacte orbicularis, mox transversa, mox ovata, in fronte saepe emarginata, vid. fig. *b*, *d*, *e*, *f*, semper compressa, densissime punctata. Apertura incompleta, v. fig. 15*d*; deltidium non vidi. Sceleton internum simplicissimum, a lamella triangulari versus frontem declivi formatum, v. fig. *a* ubi a latere inspectum est. Brachia versus cardinem connata seriem ciliarum simplicem, orbicularem antice interruptam exhibent v. f. *b*. Color testae albidus'.

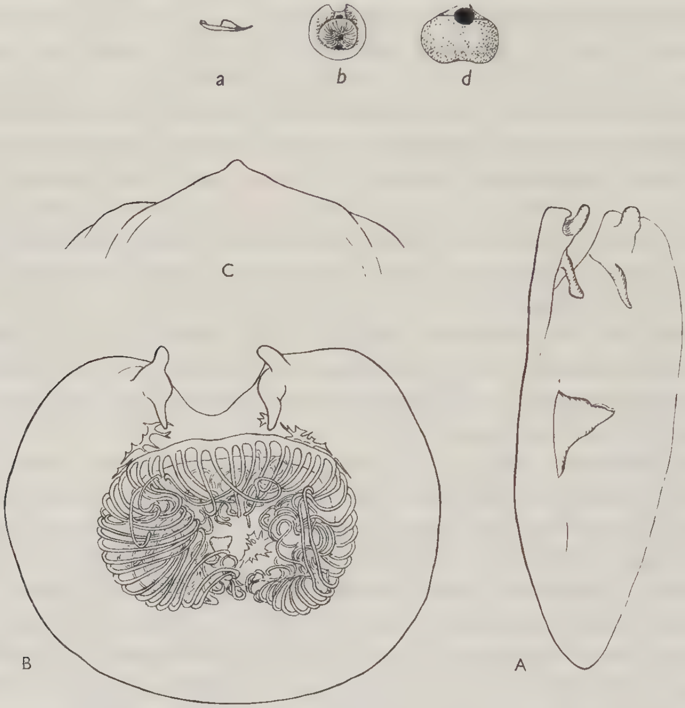


Fig. 23. A, B, C, *Platidia anomioides* of diameter 2 mm: A, side view of brachial valve; B, brachial valve with contracted schizolophe; C, beak of shell viewed ventrally (B and C are of a second individual). *a, b, d. Terebratula seminulum* Philippi of diameter 1 line, after Philippi's fig. 15*a, b, d*, for comparison with *Platidia anomioides* of the same size.

There has been argument as to whether *T. seminulum* is a *Platidia* or an *Argyrotheca* (= *Argiope*). In 1844 (p. 69) Philippi in describing *Orthis neapolitana* Scacchi (now *Argyrotheca cordata*) said 'cfr. vol. 1, p. 97, t. 6, f. 15 nomine Tereb. seminuli'. This possibly accounts for Davidson's (1852*b*, p. 514) statement in corrections to his paper earlier in the same volume (1852*a*, pp. 361–77) that 'the name *seminulum* was intended by Philippi for an *Argiope*, although his figure may have been taken from more than one shell'. In his paper (1852*a*) Davidson had evidently described and figured the *Platidia anomioides* of Philippi under the name *Morrisia seminulum* Philippi, and in 1921 (p. 332) Dall called it *Platidia seminula*, the generic name

Platidia having been proposed for the genus by Costa in January 1852, a few months earlier than Davidson's name of *Morrisia*.

Fischer & Oehlert (1891, p. 97) considered not only that *Platidia anomioides* and *Terebratula seminulum* were distinct, but that the latter probably belonged to a different generic group, perhaps to *Cistella* (= *Argyrotheca*): their opinion was apparently based on the form of the lophophore. Thomson (1927, p. 218) said that *Platidia anomioides* 'has been confused by Davidson and Dall (1921) with *Terebratula seminulum* Philippi, 1836, a species of similar external form referable to the genus *Amphithyris*'. Thomson (p. 216) separated *Terebratula seminulum* from *Platidia anomioides* because 'of the distinct character of the lophophore'. He had apparently temporarily overlooked the fact that as far as was known all the more highly organized lophophores pass through a schizolophous stage—the type of lophophore depicted by Philippi for *Terebratula seminulum*—and there was no reason to assume that *Platidia anomioides* was an exception, or that *Terebratula seminulum* seen by Philippi was necessarily in an adult state at a diameter of 1 line, that is approximately 2 mm.

Jeffreys (1878, p. 411) mentioned that according to Seguenza the young of *Platidia anomioides* is the *Terebratula seminulum* of Philippi, and this is probably what it is. *Platidia anomioides* at a shell length of about 2 mm closely resembles in shell form, triangular septum (Fig. 23A) and schizolophous lophophore (shown contracted in Fig. 23B) the figures by Philippi (1836, pl. 6, fig. 15*a*, *b*, *d*), reproduced in Fig. 23*a*, *b*, *d*, of *Terebratula seminulum* from Sicily of the same size. He clearly shows the amphithyrid foramen, not present in the genus *Argyrotheca*. The identity of the shell he figured in pl. 6, fig. 15*e*, *f*, *g*, is uncertain: it appears too elongated for even a young *Platidia*.

Unless Philippi's specimens of *Terebratula seminulum* are in existence and can be examined to determine without any doubt that they are the young of *Platidia anomioides*, then it seems best to leave that name undisturbed.

FURTHER REMARKS ON LOPHOPHORES

The brachial support is in a more advanced state in adult *Platidia anomioides* than in adult *P. davidsoni*, in that the descending branches are present and complete in the former and absent in the latter. But on the other hand the ascending branches are larger in *P. davidsoni* than in *P. anomioides*. In *P. anomioides* the descending branches, although complete, reach only to the centre of the oral disc, leaving the lateral arms unsupported except by spicules. This arrangement no doubt allows of the packing away of the lophophore in the constricted space available when the shell is closed.

The ascending branches or divergent lamellae of immature *P. davidsoni* are reminiscent of those of immature *Kraussina rubra* (Elliott, 1949) and of adult *Pumilus antiquatus* (Atkins, 1958) but differ in that they are almost horizontal and the septal pillar which bears them is considerably higher than in the two other species. Moreover, *Platidia* differs widely from the Kraussinidae in shell shape and beak characters.

Beecher (1893) gave among the diagnostic characters of the Terebratellidae King emend. 'cirri directed inwardly during larval stages', in contrast to the Terebratulidae Gray in which it was said 'cirri directed outwards in larval stages', that is in stages when the lophophore is either trocholophous or early schizolophous. Thomson (1927, pp. 180, 202) followed Beecher in making this supposed difference between the direction of the cirri or filaments in young stages one of the chief diagnostic differences between the two families, and this has been repeated by later workers.

Beecher's figures of the trocholophous and early schizolophous stages of *Dallinella* (= *Terebratalia*) *obsoleta* (1893, pl. ii, figs. 10, 11, 12) were, he stated (1895, in the legends on p. 398), 'drawn from specimens which had been dried and afterwards expanded by soaking in water, and then stained and prepared for mounting. The original proportions and relations of the parts may be therefore somewhat altered'.

In the contracted state as when the valves of the living animal are first separated, or the animal is preserved without narcotizing, the filaments in the trocholophous and early schizolophous stages in terebratellaceans are as shown by Beecher, but when the animal is feeding with the shell gaping and lophophore expanded the filaments are directed ventrally and outwards, as may be seen from the figures of *Platidia davidsoni*, and *P. anomioides* accompanying this paper and from those of *Pumilus antiquatus* (Atkins, 1958). This has also been found to be so in *Argyrotheca cordata*, *Megathyris detruncata*, *Pantellaria echinata*, *Terebratalia transversa* and *Terebratella inconspicua*, and no doubt occurs also in *Macandrevia cranium* but unfortunately the very young of this species have so far only been seen preserved. Accounts are in preparation of the growth stages of most of these species and also of those of certain other brachiopods.

THE CILIARY FEEDING MECHANISM OF *PLATIDIA*

Work on the ciliary feeding mechanism of *Platidia* was done on *P. davidsoni*, which, being dredged from only 45 to 48 fm, survived well in the laboratory tanks. After separating the valves, the lophophore soon expanded and in one, perhaps exceptional, instance remained in good condition for 10 days at temperatures of 13° to 15·6° C, with the lateral cilia beating rapidly with a good metachronal wave. This species was found to narcotize easily with stovaine one drop of 1 % stovaine being added to a watch-glass of sea water at intervals. *P. anomioides*, dredged from 400 to 405 fathoms was on the contrary difficult to deal with.

P. davidsoni usually, although not always, lifts the shell at an angle from the substratum, so that it is raised 1 to 2 mm anteriorly, preparatory to opening the valves, uncoiling the filaments, ready for feeding. It can also shift the shell a little sideways in spite of its very short stalk.

In a normally feeding animal the valves gape fairly widely to an angle of 45° or more. A large specimen of some 6 mm in length was seen to have an anterior gape of 3 to 3·5 mm, and the lower, dorsal, valve was lifted from the substratum about 2 mm in front.

The lophophore as seen through the gape has the appearance of a plectolophe, but the short spiral arm in the expanded state is almost entirely ventral to the lateral arms. The dorsal filaments of the lateral arms extend to the edge of the dorsal valve: the ventral filaments usually appear to hang down toward the lower, dorsal valve—that is they do not touch on the edge of the ventral mantle as in other brachiopods observed—so that the inhalant current is drawn in somewhat dorsally and the exhalant current leaves the shell over a wider area than in most brachiopods with plectolophous lophophores. The correct position of the filaments cannot be shown in an opened animal.

The filaments on opposite sides of the short spiral arm either touch at their tips, interdigitate, or a slight space is left between the tips, which then appear to touch on the ventral mantle.

The ciliation of the lophophore and the mantle

The body of the lophophore is entirely ciliated—except possibly for mucous cells—as indicated by the movement of particles.

On the filaments the ciliation is in three main tracts, a frontal and paired lateral tracts.

The frontal cilia (*f.c.*, Fig. 15) are about 20 μ long. They appeared to be all alike, as seen in life across the frontal surface, when an inner filament with ridged frontal surface was bent sharply outwards. These cilia, as in other brachiopods examined, seem to be capable of reversing the direction of their beat (Atkins, 1956, 1958).

The lateral, current-producing, cilia (*l.c.*, Fig. 15) are some 30 μ long. They beat across the length of the filaments from the frontal to the abfrontal surface. The metachronal wave is of the type termed dexioplectic (Knight-Jones, 1954).

Sparse abfrontal cilia occur but do not appear to be effective, as no current could be demonstrated.

The ciliary currents

On the outer abfrontal surfaces of the lateral and spiral arms currents pass toward the bases of the filaments, although no abfrontal currents on the latter could be demonstrated.

The floor, or brachial membrane, of the lateral arms has currents passing toward the median line and posteriorly in the direction of the mouth.

On the posterior and lateral regions of the large oral disc currents are directed toward the food groove. On the anterior region of the disc they are anterior in direction and particles pass off into the exhalant current.

The general direction of the currents is anteriorly on the ventral mantle. Over the middle region of the valve, that is lying to the inner side of the gonads, currents pass into an anteriorly directed current in the mid line. To the outer side of the gonads currents pass somewhat laterally and anteriorly into a submarginal anteriorly directed current. In fact over the gonads currents pass in opposite directions, toward the mid line and laterally. Currents on the dorsal mantle are similar in direction.

On the anterior body wall currents on each side of the septal pillar pass dorsally and anteriorly, and when no exhalant current is evident particles collect at the base of the septal pillar. This material would no doubt be lifted into the strong exhalant current when this is working.

In the young stages of the lophophore, trocholophous and early schizolophous, the inhalant current produced by the lateral cilia on the filaments, passes into the bell-shaped lophophore (Fig. 3B) and escapes between the filaments all around as previously described in *Terebratulina retusa* and *Pumilus antiquatus* (Atkins, 1956, 1958). As the invagination of the schizolophe deepens the inhalant current becomes divided into two and a narrow excurrent channel is present between the two lobes of the schizolophe, as in the adult *Pumilus*.

As in plectolophes generally the inhalant currents are lateral and paired and the exhalant single and median.

Platidia davidsoni was unusual in that the lateral cilia continued beating with a good metachronal wave for several days after separation of the valves. This allowed of some observations being made on them. If a filament is suddenly coiled, while the laterals are beating, they slow or stop, and as the filament is uncoiled they begin beating again. When the laterals are active there are no momentary intermissions, such as occur in lamellibranchs several times in a minute (Lucas, 1931, pp. 157–8; Atkins, 1936, pp. 295–6), although they may stop for varying periods on some filaments or parts of filaments. The lateral cilia of brachiopods will evidently remain stationary for considerable periods, for at times animals will gape without detectable currents entering or leaving the shell cavity.

On the frontal surfaces of the filaments particles are conveyed by the cilia either toward the base and eventually to the mouth, or toward the tip to be rejected to the outside of the shell, according to the size and quantity provided (Atkins, 1956).

My thanks are due to the Captain and crew of R.V. 'Sarsia' who dredged the *Platidia* and to those members of the scientific staff, particularly to

Dr A. J. Southward and Mr G. R. Foster, who were in charge of the scientific work and took care of the brachiopods for me.

Mr G. F. Elliott kindly read the manuscript. A grant from the Browne Fund of the Royal Society and a London University table made the work possible.

SUMMARY

Platidia davidsoni and *P. anomioides* dredged by R.V. 'Sarsia' have been examined living. The adult lophophore in both is shown to be a somewhat modified plectolophe, the spiral arm short with but one coil. In *P. anomioides* the brachial support consists of complete descending branches, growing from both crura and septum, and very short ascending branches arising from the apex of the septal pillar. The whole apparatus reaches only to the centre of the oral disc. In *P. davidsoni* the brachial support is much reduced: descending branches are absent; ascending branches, however, are larger than in *P. anomioides*. In both the main support of the lophophore is by spicules.

Full series of growth stages of the lophophore and its support of both species are described and figured for the first time. The growth of the lip of the food groove by extension of a small preoral lobe is described. The structure of the lophophore of *P. davidsoni* has been investigated by sectioning.

It is suggested that the *Terebratula seminulum* of Philippi 1836 is an immature stage of *Platidia anomioides*.

The value as a diagnostic character between terebratulaceans and terebratellaceans of 'filaments directed outwards' and 'filaments directed inwards up to the schizolophous stage' is commented on.

The ciliary feeding mechanism of *P. davidsoni* is briefly described.

REFERENCES

ATKINS, D., 1936. On the ciliary mechanisms and interrelationships of lamellibranchs. Pt. I. *Quart. J. micr. Sci.*, Vol. 79, pp. 181–308.

—— 1956. Ciliary feeding mechanisms of brachiopods. *Nature, Lond.*, Vol. 177, pp. 706–7.

—— 1958. A new species and genus of Kraussinidae (Brachiopoda) with a note on feeding. *Proc. zool. Soc. Lond.*, Vol. 131, pp. 559–81.

—— 1959. A new species of *Platidia* (Brachiopoda) from the La Chapelle Bank region. *J. mar biol. Ass. U.K.*, Vol. 38, pp. 133–42.

BEECHER, C. E., 1893. Revision of the families of loop-bearing Brachiopoda. *Trans. Conn. Acad. Arts Sci.*, Vol. 9, pp. 376–91.

—— 1895. The development of *Terebratalia obsoleta* Dall. *Trans. Conn. Acad. Arts Sci.*, Vol. 9, pp. 392–9.

COSTA, O. G., 1852. *Fauna del Regno di Napoli* (Not seen.)

DALL, W. H., 1921. Annotated list of the recent Brachiopoda in the collection of the United States National Museum, with descriptions of thirty-three new forms. *Proc. U.S. nat. Mus.*, Vol. 57, pp. 261–377.

DAVIDSON, T., 1852*a*. Sketch of a classification of recent Brachiopoda; based upon internal organization. *Ann. Mag. nat. Hist.*, Ser. 2, Vol. 9, pp. 361–77.

—— 1852*b*. Additions and corrections to Mr Davidson's paper on the classification of the Brachiopoda. *Ann. Mag. nat. Hist.*, Ser. 2, Vol. 9, p. 514.

—— 1852*c*. Descriptions of a few new recent species of Brachiopoda. *Proc. zool. Soc. Lond.*, 1852 (Part 20), pp. 75–83.

—— 1880. Report on the Brachiopoda dredged by H.M.S. 'Challenger' during the years 1873–1876. *'Challenger' Rep.*, Vol. 1, pp. 1–67.

—— 1887. A monograph of recent Brachiopoda. Part II. *Trans. Linn. Soc. Lond.* (*Zool.*), Ser. 2, Vol. 4, pp. 75–182.

Elliott, G. F., 1949. The brachial development of *Kraussina* (Brachiopoda). *Ann. Mag. nat. Hist.*, Ser. 12, Vol. 2, pp. 538–46.

Eudes Deslongchamps, E., 1855. On a new species of *Morrisia*. *Ann. Mag. nat. Hist.*, *Ser.* 2, Vol. 16, pp. 443–4.

Fischer, P., 1872. Brachiopodes des côtes océaniques de France. *J. Conchyliol.*, T. 20, pp. 160–4.

—— 1873. Faune conchyliologique marine du department de la Gironde et des côtes du sud-ouest de la France. *Act. Soc. linn. Bordeaux*, T. 29, (Sér. 3, T. 9), pp. 193–255.

Fischer, P. & Oehlert, D.-P., 1891. Brachiopodes. *Expéd. sci. Travailleur et Talisman*, 140 pp.

Hancock, A., 1858. On the organization of the Brachiopoda. *Phil. Trans.*, Vol. 148, pp. 791–869.

Jeffreys, J. G., 1878. On the Mollusca procured during the 'Lightening' and 'Porcupine' Expeditions, 1868–70. (Part 1). *Proc. zool. Soc. Lond.*, 1878, pp. 393–416.

Knight-Jones, E. W., 1954. Relations between metachronism and the direction of ciliary beat in Metazoa. *Quart. J. micr. Sci.*, Vol. 95, pp. 503–21.

Lucas, A. M., 1931. An investigation of the nervous system as a possible factor in the regulation of ciliary activity of the lamellibranch gill. *J. Morph.*, Vol. 51, pp. 147–93.

Massy, A. L., 1925. The Brachiopoda of the coasts of Ireland. *Proc. R. Irish Acad.*, Vol. 37, pp. 37–46.

Morse. E. S., 1871. On the early stages of *Terebratulina septentrionalis* (Couthouy). *Ann. Mag. nat. Hist.*, Ser. 4, Vol. 8, pp. 414–27.

Percival, E., 1944. A contribution to the life-history of the brachiopod, *Terebratella inconspicua* Sowerby. *Trans. roy. Soc. N.Z.*, Vol. 74, pp 1–23.

Philippi, R. A., 1836. *Enumeratio Molluscorum Siciliae*. Vol. 1.

—— 1844. *Enumeratio Molluscorum Siciliae*, Vol. 2.

Prenant, M., 1928. Notes histologiques sur *Terebratulina caput-serpentis* L. *Bull. Soc. zool. Fr.*, T. 53, pp. 113–25.

Richards, J. R., 1952. The ciliary feeding mechanism of *Neothyris lenticularis* (Deshayes). *J. Morph.*, Vol. 90, pp. 65–92.

Senn, E., 1934. Die Geschlechtsverhältnisse der Brachiopoden, im besonderen die Spermato- und Oogenese der Gattung *Lingula*. *Acta zool.*, *Stockh.*, Bd. 15, pp. 1–152.

Thomson, J. A., 1927. Brachiopod morphology and genera (recent and tertiary). *N.Z. Bd Sci. Art*, Manual 7.

Williams, A., 1956. The calcareous shell of the Brachiopoda and its importance to their classification. *Biol. Rev.*, Vol. 31 pp. 243–87.

J. mar. biol. Ass. U.K. (1959), 133–142
Printed in Great Britain

A NEW SPECIES OF *PLATIDIA* (BRACHIOPODA) FROM THE LA CHAPELLE BANK REGION

By D. Atkins, D.Sc.
From the Plymouth Laboratory

(Text-figs. 1–8)

A new species of *Platidia* (Brachiopoda, Platidiidae) was dredged by R.V. 'Sarsia' in November 1956 in the La Chapelle Bank region, attached to the dead part of the coral *Anisopsammia rostrata* (Pourtales).[1] Eight entire specimens and two smashed were dredged at position 47° 30′ N., 7° 20′ W. at a depth of 870 to 970 fathoms, and one at position 47° 38′ N., 7° 28′ W. at 710 to 750 fathoms. All, except the two smallest, were a reddish brown, the coloration being due to a deposit of manganese oxide: the natural colour is a yellowish white. The dead coral was almost black with the same deposit (see Southward, 1958, p. 644).

Some individuals were transversely oval, others somewhat rectangular and irregular in shape, according to the surface of attachment (Fig. 1). The *Anisopsammia* was fairly free of encrusting organisms but the polyps are closely spaced and therefore apt to interfere with the regular spreading of the closely attached *Platidia*.

The new species of *Platidia* was difficult to deal with. As it was from deep water it was brought back in a refrigerator at a temperature of 5°–7° C: specimens then went into laboratory tank water of 13° C. It has been found that brachiopods generally cannot withstand sudden changes of temperature, and it was this change in temperature which no doubt adversely affected the specimens, most of which showed separation of the cells of the epithelium of the filaments after a few days. Those opened the day after arrival in the laboratory did not extend the filaments on the anterior region of the lateral arms, although the posterior and lateral filaments and those on the posterior extremities of the lateral arms expanded well. This continued contraction of the filaments of the anterior region of the arms may be dependent in some way on the fact that the arms remained depressed and did not raise themselves as they do under similar conditions in *P. davidsoni* and *P. anomioides*. Attempts were made to narcotize the animals with 1 % stovaine and with 7 % magnesium chloride, a drop added at intervals to a small dish or watch glass of sea water. The latter acted rather the better, but results were not good. The specimen with most expanded lophophore is shown in Fig. 5: it is not fully expanded. This specimen left in cedar wood oil for a few months suffered severe deterioration, the tissue becoming extremely brittle, and fragmenting.

---

[1] Identified by Dr A. J. Southward.

All figures were drawn with the aid of a camera lucida.

Platidia annulata sp.nov.

Description

The general shape of the shell is that typical of the genus *Platidia* (Fig. 1, A, B). The pedicle is short, and the dorsal or brachial valve is closely applied to the substratum; it is generally approximately flat, while the ventral valve is gently domed. So far as can be judged by the few specimens obtained, this is a small species. The three largest individuals had the following measurements: (*a*) length 4·7 mm, width 4·2 mm; (*b*) length 4·2 mm, width 4·3 mm; (*c*) length 3·7 mm, width 4·7 mm. This is approximately the size of *P. anomioides* (Philippi) found to the south-east of the La Chapelle Bank area (Atkins, 1959).

The test is smooth, with growth lines discernible, and is conspicuously punctate. As in *P. anomioides* and *P. davidsoni* the punctae appear to be smaller on the dorsal than on the ventral valve, probably because the shell is thinner. The beak is very short, rostrum apicate, foramen amphithyrid. The deltidial plates are narrow. The pedicle collar is short, sessile and not as conspicuous as in *P. anomioides* and *P. davidsoni*. Running anteriorly from the pedicle collar is a slight median elevation. The hinge teeth have very feeble dental plates as in the other two species. Fig. 2 shows the beak region at three sizes.

In the dorsal valve the inner socket ridges are prominent; the crural bases arise from them. A high septal pillar is present posteriorly. In adults the pillar is continued posteriorly by a septum gradually decreasing in height to a mere ridge on the foraminal margin. The crura are long, the crural processes short, but distinct. The descending branches are quite stout for the size of the shell and widen broadly at their insertion high on the septum, up which they run obliquely to become attached to the sides of the ring formed by the ascending branches. The tips of the short ascending branches are connected by a transverse bar, so that a ring is formed over the septum (Fig. 3). The brachial support is thus more completely developed than in any other species of *Platidia* described, but in spite of this the descending branches reach only to the centre of the oral disc.

The lophophore may be considered a modified plectolophe with an extremely small spiral arm, or even as a modified zugolophe (Fig. 5): it is possible that if *P. annulata* grows to a larger size than seen so far, the spiral arm may also be correspondingly developed. A greater area of the ventral surface of the oral disc is free from spicules than in either *P. anomioides* or *P. davidsoni*: they are present over an area anterior to the transverse bar of the loop (Figs. 5, 7A).

P. annulata is especially noticeable for the long, closely set mantle setae, reaching a length of at least 2 mm; considerably longer than those of *P. anomioides* and *P. davidsoni*. The setae in the specimens seen were dark and iridescent, but this was probably due to a deposit present also on the shells

The flesh of specimens was pale coloured: this may have been because the gonads were not fully developed.

As alcohol, neutral formalin and cedar wood oil have severally caused deterioration of the few specimens obtained, no single one has been chosen as the holotype. The adult specimens which may be considered as syntypes have been deposited in the British Museum (Natural History) and given the following numbers: ZB. 2910–16, all at 870 to 970 fm.; ZB. 2917 at 710–15 fm.

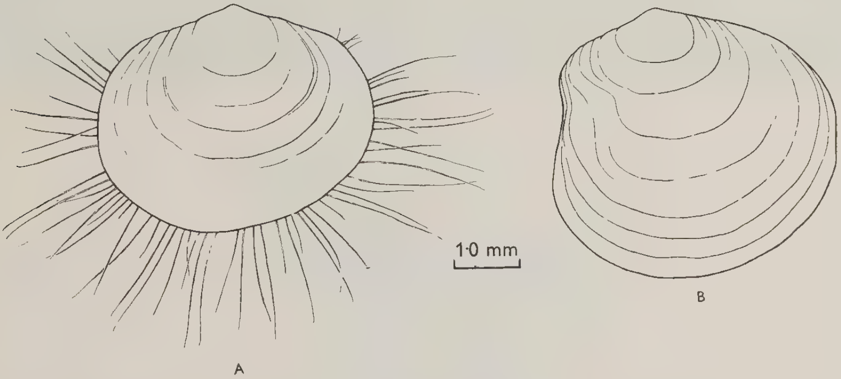


Fig. 1. *Platidia annulata.* A, specimen of regular, transversely oval, shape; mantle setae shown: shell length 3·5 mm, width 4·2 mm. B, specimen of irregular shape; mantle setae omitted: shell length 4·2 mm, width 4·3 mm.

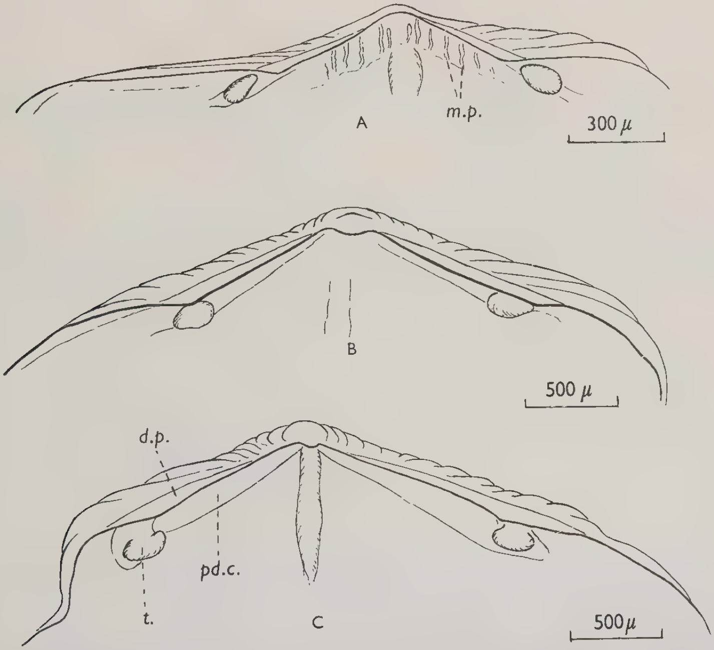


Fig. 2. *Platidia annulata.* Beaks of three individuals of different size. A, length 2·2 mm, width 2·4 mm; B, length 4·2 mm, width 4·3 mm; C, length 4·7 mm, width 4·2 mm. *d.p.*, deltidial plate; *pd.c.*, sessile pedicle collar; *m.p.*, mantle pits in pedicle collar; *t.*, tooth.

THE ADULT LOPHOPHORE

Material for the study of the growth stages of the lophophore was not available. Two small specimens, one 2·1 mm long and 2·2 mm wide, and the other 2·2 mm long and of irregular shape, were obtained. The first will be described later as it is not altogether certain that it belongs to this species, indeed it cannot be certain that the second does either, although both were

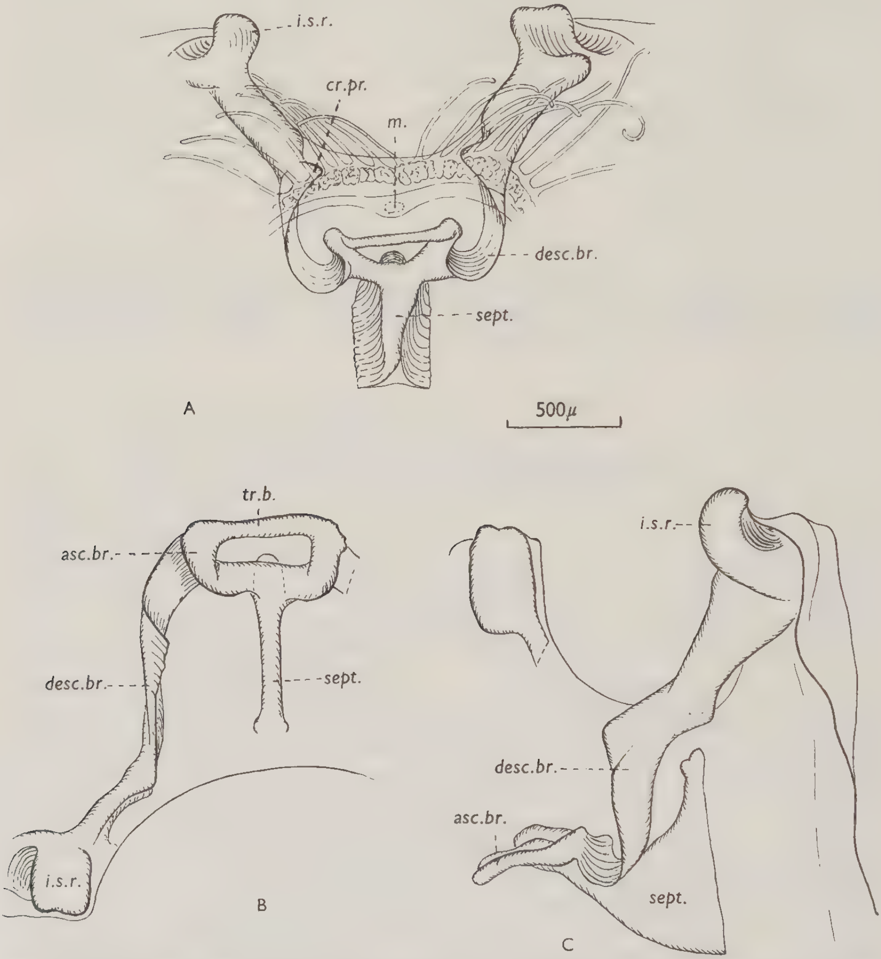


Fig. 3. *Platidia annulata.* The brachial support of (A) individual 3·5 mm long and 4·2 mm wide; (B and C) individual 4·7 mm long and 4·2 mm wide. A, ventral view with posterior filaments indicated; B, posterior view, with brachial valve standing on its anterior edge. The left descending branch broke and fell away before the drawing was made. C, side view of the same. *asc.br.*, ascending branch; *cr.pr.*, crural process; *desc.br.*, descending branch; *i.s.r.*, inner socket ridge; *m.*, position of mouth covered by lip; *tr.b.*, transverse band of the loop; *sept.*, septum.

found in the haul which contained the adults. The second specimen was an empty shell although still attached to the coral; it had probably died since collection. The septal pillar was high, not triangular, with minute triangular prongs at the apex. Crura and crural processes were present; descending branches from the crura were short and their anterior ends were wanting (Fig. 4). In *P. davidsoni* at a corresponding size the septum is low, with almost horizontal lamellae at its apex: crura are absent (Atkins, 1959).

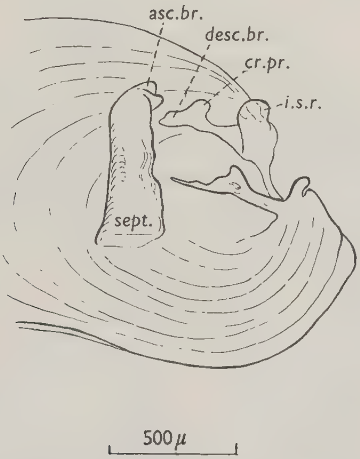


Fig. 4. *Platidia annulata.* Brachial support of immature individual of irregular shell shape; length 2·2 mm. *asc.br.*, ascending branch; *cr.pr.*, crural process; *desc.br.*, descending branch; *i.s.r.*, inner socket ridge; *sept.*, septum.

P. anomioides at about this size has the septum a broadly based triangle, and although long crura are present, descending branches have not yet grown from them. At a slightly larger size, however, the septum may become taller and less triangular in shape (Atkins, 1959).

The largest individual of *P. annulata* obtained has a shell length of 4·7 mm and width of 4·2 mm. At this size the lophophore was in about the same condition as in the individual figured (Fig. 5) of length 3·5 mm and width 4·2 mm; there was little development of the spiral arm and the recurved portion of the lateral arms was short.

Some eighteen to twenty pairs of filaments behind the mouth are in single series and have ridged frontal surfaces.

In the adult the descending branches, although complete, reach only to the centre of the oral disc, and together with the septum, ascending branches and transverse bar support no more than the posterior region of the lophophore to about the centre of the oral disc (see Figs. 5, 6). The individual of which the lophophore is figured (Fig. 5) has a wide base to the septal pillar, the anterior face of which is somewhat asymmetrical (Fig. 3 A).

In *P. annulata* a lesser area of the oral disc is covered with spicules than in either *P. anomioides* or *P. davidsoni* as may be seen from Fig. 7.[1] A band at the bases of the filaments, narrow behind the mouth, widens laterally as it passes on the outer side of the descending branches into the lateral arms (Fig. 5). The abfrontal surfaces of these are almost covered with large spicules, only a small central area in each being free. The floor of the lateral arms, or brachial membrane, seems to be without spicules, except perhaps for a narrow band at the bases of the filaments. A ventral area of spicules beginning at the transverse band extends to the anterior edge of the oral disc; laterally it joins the bands just described.

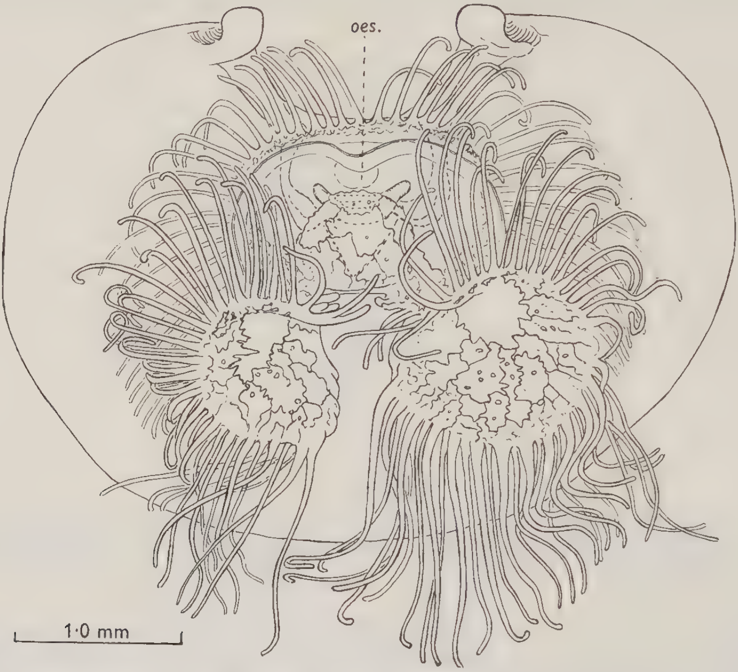


Fig. 5. *Platidia annulata.* Shell length 3·5 mm, width 4·2 mm. Brachial or dorsal valve with the lophophore not fully expanded: drawn narcotized. Brachial support, indicated by broken lines, added after clearing in cedar wood oil. *oes.*, position of oesophagus.

The outer filaments with grooved frontal surfaces are heavily spiculated, the inner ones, with ridged frontal surfaces less so. The spicules are broad bands with granulated surface such as are present in the filaments of the other two species.

The lips of the food groove are without spicules.

---

[1] In larger specimens of *P. anomioides* an even smaller area of the oral disc is left naked than shown in the figure.

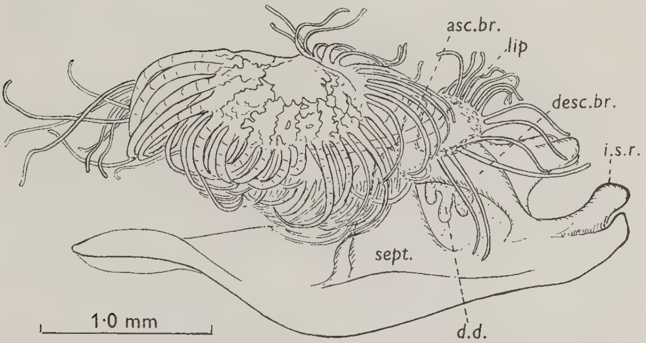


Fig. 6. *Platidia annulata.* Same individual as in Fig. 5 shown in side view to show position of the lophophore with regard to the descending branches and the septal pillar. The lophophore is somewhat depressed and contracted anteriorly. *asc.br.*, ascending branch; *d.d.*, digestive diverticula; *desc.br.*, descending branch; *i.s.r.*, inner socket ridge; *lip*, lip of food groove; *sept.*, septum.

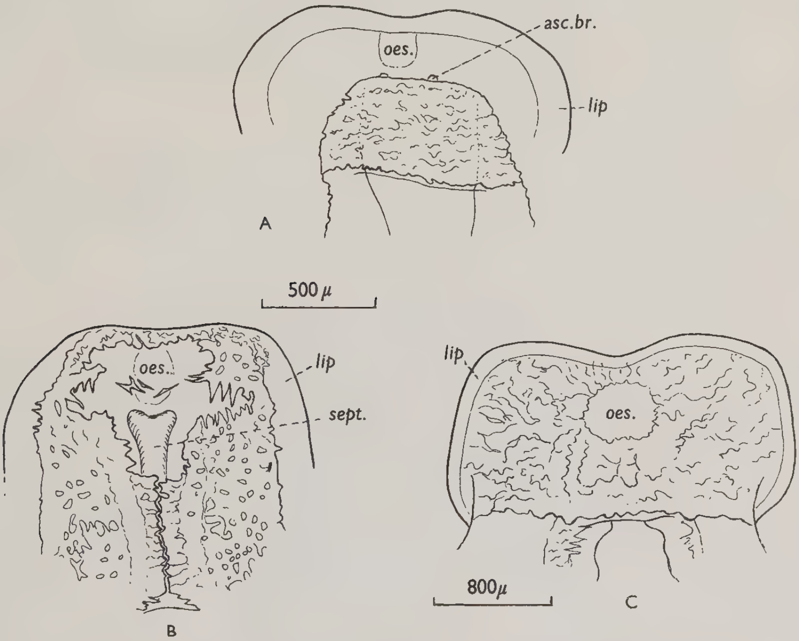


Fig. 7. Oral discs to show the distribution of spicules on the ventral surface of A, *Platidia annulata* of shell length 4·2 mm, width 4·3 mm; B, *P. anomioides* of shell length 3·9 mm, width 4·1 mm (at a larger size an even smaller area of the disc is naked); C, *P. davidsoni* of shell length 6·5 mm, width 8·1 mm. *asc.br.*, tip of ascending branch; *lip*, lip of food groove; *oes.*, position of oesophagus; *sept.*, septum. A and B are drawn to the same scale.

Description of small Platidia annulata (?) *of shell length* 2·1 *mm and width* 2·2 *mm*

Although of small size this individual was a male with small gonad containing tailed sperm. The lophophore might be described as late zugolophous (Fig. 8), but the lateral lobes or arms in the contracted state are so arranged that the brachial membrane faces dorsally, being almost parallel with the valve floor, instead of facing laterally. In this it resembles the adult lophophore and is almost in the adult state. The two growing regions are separated. The brachial support consists of a septum bearing two short ascending branches, without transverse band, and at a more dorsal level two small processes, the anterior ends of the descending branches: the posterior ends arising from the crura are short. The descending branches thus arise from both crura and septum as in *P. anomioides* (Atkins, 1959). It may be assumed that the brachial support is not yet fully formed.

On the oral disc a band of spicules, narrow behind the mouth, widens as it passes on either side of the septum into the lateral arms. This band seems relatively more developed than in the adult lophophore of *P. annulata*, while the area of spicules extending on the ventral surface from the transverse band to the anterior edge of the oral disc is wanting. It is not impossible that the latter may develop at a larger size.

The number of filaments behind the mouth with ridged frontal surfaces is about 19 pairs as in adult specimens of *P. annulata*. These, as is usual in brachiopods, are continued in the same line by grooved filaments, but the inner alternating row of filaments could not be distinguished, possibly because of the strongly contracted state of the lophophore in an animal preserved on board R.V. 'Sarsia' without narcotizing.

This small individual cannot certainly be identified with *P. annulata* although found in the same habitat and in the same haul. Unfortunately no especial attention was paid at the time to the length of the mantle setae of this particular specimen, and these have now been shed. The brachial support not being fully developed does not assist in identification, except that it excludes *P. davidsoni*. This specimen could be the young of *P. annulata*: although the arrangement of the spicules on the oral disc is not what might have been expected. It would not seem to be a young stage of *P. anomioides* for it is considerably more advanced in stage of lophophore, brachial support and gonad than is *P. anomioides* at about the same size; compare Fig. 8 with Fig. 23A, B in Atkins, 1959 (p. 126 of this Journal).

REMARKS

Perhaps the characters by which *P. annulata* is most easily separated from *P. anomioides* and *P. davidsoni* are (1) the loop over the septum (Fig. 3); (2) the small area of the oral disc covered by spicules (Figs. 5, 7); and (3) the long mantle setae up to at least 2 mm long (Fig. 1). There is also a difference in the number of filaments in single series behind the mouth: in *P. annulata* the number is 18 to 20 pairs, in *P. anomioides* 25 to 26 pairs, and in *P. davidsoni* 16 to 17 pairs (Atkins, 1959).

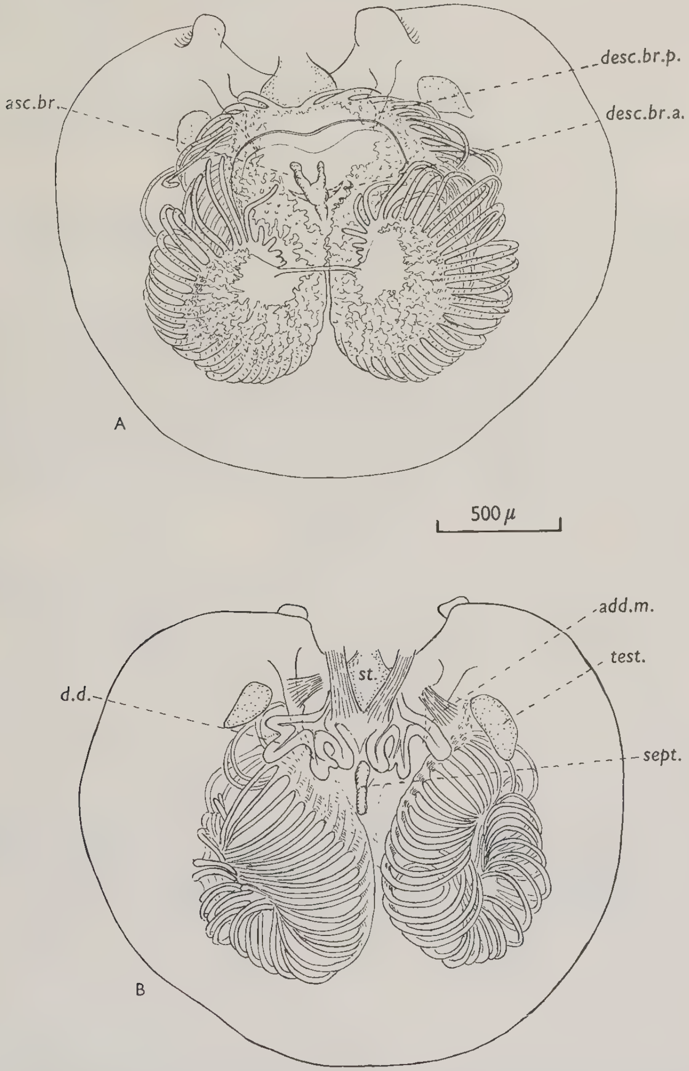


Fig. 8. *Platidia annulata* (?): shell length 2·1 mm, width 2·2 mm. Brachial valve with lophophore. A, ventral view: the distance apart of the two growing regions is to be noted. B, dorsal view of lophophore and body seen through the shell after staining and clearing in cedar wood oil. *add.m.*, adductor muscle; *asc.br.*, ascending branch; *d.d.*, digestive diverticula; *desc.br.a.*, anterior, and *desc.br.p.*, posterior ends of descending branch; *sept.*, septum; *st.*, stomach; *test.*, testis.

The umbonal region of *P. annulata* (Fig. 2) resembles that of *P. anomioides* more than that of *P. davidsoni*.

Thomson (1927, p. 219) remarked that 'Beecher placed the genus (i.e. *Platidia*) in the Dallininae, comparing the loop with the earliest loop stages of *Macandrevia* and *Dallina*. A distinction, however, is that in *Platidia* the ascending branches are not united to form a hood or ring over the septum.' They are now known to be so connected in *P. annulata*, but even so it is most doubtful whether *Platidia* could be placed in the Dallininae as the descending branches from the loop arise from both crura and septum (Atkins, 1959), whereas it is said to be characteristic of the Dallininae that they arise from the crura only (Thomson, 1927, p. 234).

Although of different form and different development the loop of *P. annulata* is as short in relation to the lateral arms of the lophophore as that of the short-looped terebratulaceans.

My thanks are due to the Captain and crew of R.V. 'Sarsia' who dredged the new species, and particularly to Dr Eve C. Southward who picked out the coral bearing the brachiopods and took care of them on board. Mr G. F. Elliott kindly read the manuscript. A grant from the Browne Fund of the Royal Society and a London University table made the work possible.

SUMMARY

A new species of *Platidia*, *P. annulata*, has been found and described. It is characterized by having a complete ring over the septum, a small area only of the oral disc covered by spicules, and long mantle setae.

REFERENCES

ATKINS, D. 1959. The growth stages of the lophophore of the brachiopods *Platidia davidsoni* (Eudes Deslongchamps) and *P. anomioides* (Philippi), with notes on the feeding mechanism. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 103–132.

SOUTHWARD, A. J. & SOUTHWARD, EVE C., 1958. On the occurrence and behaviour of two little-known barnacles *Hexelasma hirsutum* and *Verruca recta*, from the continental slope. *J. mar. biol. Ass. U.K.* 37, pp. 633–47.

THOMSON, J. A., 1927. Brachioped morphology and genera ((Recent and Tertiary). *N.Z. Bd Sci. Art*, Manual 7.

J. mar. biol. Ass. U.K. (1959) 38, 143–152
Printed in Great Britain

MOVEMENT RECEPTORS IN DECAPOD CRUSTACEA

By C. A. G. Wiersma[1]

California Institute of Technology.
From the Plymouth Laboratory and Department of Zoology, Cambridge

(With Text-figures 1–3)

The sense organ of *Carcinus maenas* located in the joint between the dactylo- and propodite possesses several types of sense cells (Wiersma & Boettiger, 1958). The most interesting types react to unidirectional displacement of the dactylopodite with equal sensitivity over the total possible arc of movement. Velocity has little influence on the discharges of the movement fibres since the maximum rate of firing is reached at a speed which is relatively little faster than the threshold. Fibres with marked differences in threshold are present for both extension and flexion. Other units in the organ react like conventional stretch receptors and give continuous discharges as long as the joint is near one of the extreme positions. Again different units are present for extension and for flexion. These position fibres are on the whole smaller than the movement fibres and their cell bodies have a more peripheral location in the organ.

Organs similar to this PD-organ of *Carcinus* are known to be present in other joints and in other species (Alexandrowicz, 1958, and personal communication). The purpose of the present investigation was to determine their functions and particularly to find out whether they possess movement receptors.

METHODS AND MATERIAL

Unit analysis of fibres reacting to manipulation of specific joints was made by preparing single fibres or small bundles according to the methods described elsewhere (Wiersma & Boettiger, 1958). In this investigation all fibres were prepared from the nerve or nerves in the meropodite. As it was not the purpose to study the relation between speed of movement and the discharge rate in the movement fibres, but merely to demonstrate their presence or absence, manual manipulation of the joints sufficed. For registration purposes a simple mechanical device was used which correlated the movements of the carpo-dactylopodite joint with the displacement of the second beam of a Cossor oscilloscope. For this purpose the knob controlling the vertical position of this beam was replaced by a brass pulley over which a thread without end was looped twice. The lower thread was similarly looped over the propodite at a convenient distance from the joint and continued to a pulley located on the other side of the preparation and then back to the brass pulley. By adjusting the tension in the thread and the place of the loop around the propodite, it was possible to establish a satisfactory relation between the vertical displacement of the beam and the maximum arc of movement of the joint. Since the brass knob was moved by hand, the displacement was not smooth, with resulting irregularities in the discharges, especially from the most sensitive movement receptors. But the method permitted clear distinction between the properties of most fibres.

---

[1] John Simon Guggenheim Memorial Fellow.

In many preparations the nerve fibre bundles from which proprioceptive responses were obtained were subsequently traced to the organs from which they originated. To this end staining with methylene blue and additional exposure of the nerve bundles and organs were used.

The animals investigated were *Carcinus maenas*, *Maia squinado*, *Eupagurus bernhardus*, *Homarus vulgaris* and *Palinurus vulgaris*. The experiments on the latter four species were performed at Plymouth. In each animal the organs of the walking legs were investigated. Sea water was used as a physiological solution. For *Maia* a special solution was also tried, but no noticeable difference was observed, possibly because each experiment continued for at most two hours.

RESULTS

The PD-organs of various species

A comparison of the responses from the PD-organs of the other species with those of *Carcinus*, described elsewhere (Wiersma & Boettiger, 1958), shows that they are greatly similar. In all cases a small bundle of nerve fibres can be isolated in the meropodite, containing axons responding to different manipulations of the joint. In *Palinurus*, especially, fibres in another bundle also respond. It could be shown that these responses do not originate in the organ but from sense cells of hairs located on the distal part of the propodite, which are bent by the dactylopodite when it nears the extreme flexed position. These hairs form only part of the cluster present and on touch the signals in their fibres do not differ from those which are not stimulated when the dactylopodite is bent. It seems therefore unlikely that they have a proprioceptive function.

The four main types of fibres, opening and closing movement fibres, opened and closed position fibres, were regularly obtained from the PD organs of all genera with the exception of *Homarus*. In the PD nerve bundle of the latter closed position fibres were usually absent. Since all but the fourth walking legs are either chelated or subchelated in this species, it seemed possible that the stop provided by the propodite might be responsible. Therefore the fourth leg, in which the dactylopodite can move through as large an arc as in other genera, was especially used, but tonically firing closed position fibres were here as rare as in the other legs.

For a few preparations from different species, a count was made of the number and type of fibre which could be prepared from one PD nerve bundle. Because of damage such counts will not show the actual number of active units, but they will provide an approximation of the numerical relation of the different fibre types. In Table 1 the results are shown.

TABLE 1. NUMBERS OF REACTING AXONS PREPARED IN THE PD-NERVE BUNDLES OF VARIOUS SPECIES

	Movement		Position	
	Opener	Closer	Opened	Closed
Carcinus	4	5	6	3
Maia	4	4	1	1
Homarus	6	3	6	0
Palinurus	5	6	15	5

The large number of fibres obtained from *Palinurus* resulted from (1) individual fibres being relatively larger and thus more readily prepared, and (2) there being a larger number of nerve cells in the PD-organ than in the other genera. The number of movement fibres for the opposite directions seems about equal in all genera. These counts do not show that here, as in all other joint organs, the number of position fibres is larger than that of the movement fibres. This is due to the smaller size of the position fibres, which makes them harder to prepare, and explains why in the preparation of *Maia* presented only a single one for each class was found, since in *Maia* the nerve fibres are smaller than in other genera used.

By staining the organs with methylene blue, it was observed that the elastic strand, which is so distinct in *Carcinus*, is a much more diffuse structure, and that the cell bodies are spread farther apart. This feature and the fact that the nerve bundle close to the organ is enveloped in a strong sheath have prevented splitting of subbundles, which showed in *Carcinus* the localization of the different reaction types. Since in all organs the larger cells are located at the proximal end and the signals of movement fibres are regularly larger than those of position fibres it is likely that a similar distribution is present in all organs.

The proprioceptor organs in the carpo-propodite joint

In this joint at least two separate organs are present in all genera investigated. Information about their occurrence has been given by Alexandrowicz (1958).[1]

---

[1] Alexandrowicz (personal communication) has suggested that receptors belonging to this system might be designated by the first letters of the segments between which they are situated, viz. PD—the organ between the propodite and the dactylopodite, CP—that between the carpopodite and the propodite, etc. When two organs differing somewhat in their structure are present near to one another, as at the mero-carpopodite and carpo-propodite joints, they may be distinguished by numerals, viz. MC 1, MC 2 and CP 1, CP 2 respectively.

In *Carcinus* the organs were readily found, at first by tracing back to their origin the bundles in which proprioceptive impulses were present. CP 1 (Fig. 1A) runs from the tendon of the bender muscle (productor propoditis) to the joint membrane. CP 2 (Fig. 1B) originates on the tendon of the stretcher muscle (reductor propoditis) and bridges the joint, being attached to the inner wall of the propodite. In *Carcinus* a clear elastic strand is present in both; the one of CP 2 resembles that of the PD organ closely, but the strand of CP 1 is broader and flatter and its origin on the bender tendon less distinct.

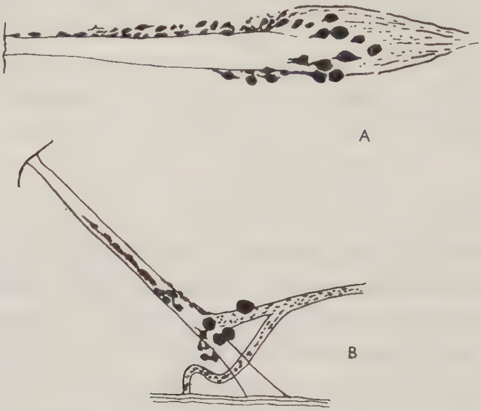


Fig. 1. *Carcinus maenas*. A, Carpo-propodite sense organ (CP 1), showing the arrangement of the nerve cells around the rather broad and proximally indefinite elastic strand. On the right the large cells of the proximal part, along the upper (outer) side the more peripheral continuation of small cells. After microphotograph of Rongalit methylene-blue stained preparation. B, CP 2 organ. Note the distinct elastic strand and the large nerve cells at the proximal end, which are in contrast to the peripheral smaller cells, loosely attached. Separate nerve branch disappears on the tendon of the stretcher muscle.

CP 1 has many more nerve cells than CP 2 and they are clearly arranged in two main rows, one on each side of the strand. The outer row continues farther peripherally than the inner, and in both there is a rather regular decrease in cell size towards the peripheral end. In CP 2 of *Carcinus* a remarkable feature is the location of the large cells at the proximal end, which are here only loosely connected to the elastic strand and sometimes a considerable distance from it.

In *Carcinus*, as in all other genera but *Palinurus*, the two nerve bundles from the two organs run well separated in the main nerve trunk in the meropodite. In *Palinurus* they often appeared to run close together, which prevented assigning them to each organ without subsequent tracing. In *Homarus* and *Eupagurus*, where a thicker and thinner nerve bundle are present, both CP bundles are located in the thicker nerve.

In all genera fibres reacting to unidirectional movement as a stimulus have been found in the nerve bundles of both CP 1 and CP 2. Regularly several fibres differing in threshold but all indicating the same direction of movement are present in each bundle. Fig. 2A illustrates a rather sensitive movement fibre of *Homarus*. The most sensitive movement fibres are not suitable for recording since very slight changes in speed will greatly influence the discharges. In Fig. 2B medium sensitive movement fibres from *Maia* are shown, one for each direction, whereas insensitive fibres are represented by one from *Eupagurus* in Fig. 2C.

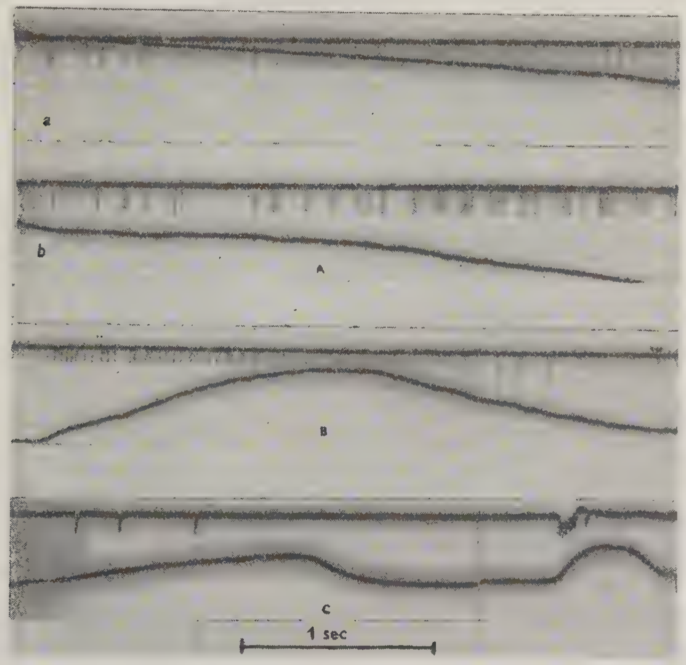


Fig. 2. A, response of sensitive flexor movement receptor from CP 1 of *Homarus*. *a*, start of the movement from complete extension; *b*, continuation to almost complete flexion. The irregularities in the discharge rate are mainly caused by those of the movement. B, a medium sensitive extensor and a flexor movement fibre from CP 1 of *Maia*, the first with the smaller signal discharging during a complete extension of the propodite, the second during flexion. C, insensitive extension movement fibre from CP 1 of *Eupagurus bernhardus*. Two complete extensor and flexor movements, the second faster. Note the limited number of impulses this fibre can maximally produce. Time-scale 1 sec. during one extension.

Besides movement fibres, position fibres are invariably present in both CP nerve bundles. These showed no properties other than those discussed for the PD-organ of *Carcinus* (Wiersma & Boettiger, 1958). Types which reacted only on position changes and types in which the frequency increased temporarily above that maintained during displacement were both noted.

In contrast to the PD-organ the CP-organs do not always respond to both movement directions. CP 1 resembles PD in this respect more than CP 2.

The latter may completely fail to respond to either movement or position in one direction, whereas CP 1 usually shows only a pronounced preference for one side. The CP-organs of *Carcinus* have been more closely studied in this respect but the phenomenon was present in all genera studied.

The dominant responses are extension for CP 1, flexion for CP 2. Large specimens invariably showed this preference for one direction, whereas in younger ones it was less pronounced though present. Counts made of the number of active fibres which could be prepared from the two bundles illustrate this feature (Table 2). In the last preparation of this table a reversal of the dominance appears, and subsequent tracing of the bundles to the organ showed it to be real, though it is the only instance in any genus encountered during this investigation.

TABLE 2. NUMBER OF ACTIVE UNITS PREPARED IN THE CP 1 AND CP 2 NERVE BUNDLES IN FIVE LEGS (A–E) OF *CARCINUS*

		Movement		Position	
		Extensor	Flexor	Extended	Flexed
A	CP 1	3	1	3	2
	CP 2	0	4	0	2
B	CP 1	4	0	3	1
	CP 2	0	5	0	2
C	CP 1	6	3	5	2
	CP 2	0	5	1	3
D	CP 1	4	0	6	0
	CP 2	1	4	0	5
E	CP 1	1	5	1	6
	CP 2	5	0	4	1

It should be noted that the dominant response from an organ occurs during the time that the elastic strand is relaxed by the movement, whereas the opposite might have been expected. That there was actual relaxation has been confirmed by studying the changes of exposed organs during the movements.

In several preparations, especially from *Palinurus*, fibres with a reaction to both movement and position were found. They did not occur in every preparation and were always accompanied by more conventional fibres. Sometimes there would be several of them in one bundle. Fig. 3A shows a type rather common in *Palinurus*, whereas Fig. 3B shows a rarer one for *Homarus*. As will be discussed, they may represent more or less abnormal sense cells. In *Eupagurus* a single fibre was found which reacted equally well to movement in either direction over the full range of the possible movement arc. This remarkable fibre is the only one so far encountered for which the name 'vibration fibre' would be appropriate.

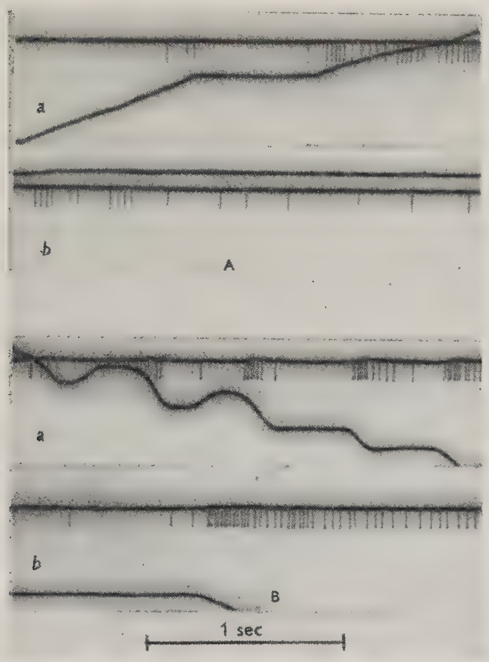


Fig. 3. A, receptor with intermediate properties from *Palinurus*. Insensitive to movement during the first part of the movement arc (*a*) it becomes quite sensitive for the second part and starts to respond to position though the maintained discharge rate remains low even in the extreme position (*b*). B, receptor with intermediate properties from *Homarus*. Movement sensitivity present throughout the arc from the extreme extended (*a*) to the extreme flexed position. In the latter it gives after the movement discharge a constant discharge at 20–25 impulses per sec. (*b*). The sections *a* and *b* are not continuous.

MC-receptors

For this joint there are again two main organs present, MC 1 which closely resembles CP 1 in structure and MC 2 which resembles CP 2. At least in *Carcinus* MC 1 is attached to the tendon of the accessory flexor muscle of the carpopodite (adductor carpi), and MC 2 is attached close to the tendon of the main flexor muscle, and, in contrast to MC 1, crosses the joint to be inserted on the inner side of the carpopodite. By exposing the nerve in the proximal part of the meropodite, leaving the distal part intact, a nerve bundle reacting to manipulation of the joint could be found for each organ. These bundles are widely separated. The bundle to MC 1 is easy to find as it usually leaves the main nerve trunk early in the meropodite.

In *Carcinus* the responses obtained from the nerve bundle of MC 2, as well as fibre analysis, showed that the organ typically, but not exclusively, signals joint flexion, whereas MC 1 has a preponderance of extensor movement and position fibres. In some preparations of *Carcinus*, no signals could be obtained from the MC 1 bundle and in *Homarus* this happened often. The reason for such failure may be that the preparation of the nerve in the meropodite usually involves cutting the tendon of the accessory flexor muscle and hence interference with the natural support of the organ. In other genera, especially *Homarus*, the preference of MC 2 to react to flexion was also noted.

'*Slit sensilla*' *of* Homarus

Dr Alexandrowicz kindly called my attention to the presence of a number of pits, located just distally to the ischio-meropodite, mero-carpopodite and carpo-propodite joints of *Homarus* and *Palinurus*. It was observed that the location of these pits is such that they are covered by their joint membranes when the joint is moved maximally to the side on which they occur. The 'sensilla' are places where the cuticle is pierced by fine channels to which dendritic extensions of sensory cells run. These nerve cells are associated with those of Barth-organ, MC2 and CP2. For the crayfish, *Potamobius torrentium*, Barth (1934) has described a similar arrangement of the innervation of 'Sinneskegel', but externally these 22–23 sensilla do not appear to be distinct, though they are located in the same general area as the 'slit sensilla' of the above-mentioned animals.

In *Homarus*, but not in *Palinurus*, it was possible to record impulses from small prepared subbundles when the pits on the propodite were touched. The reacting nerve fibres did not run together with the CP 2 proprioceptor fibres, but formed part of a larger bundle consisting of fibres responsive to touch of hairs on the ventral and proximal aspect of the propodite. This bundle was located near the CP 2 bundle. By tracing the two bundles forward it was shown that the bundle of hair fibres had no connexion with the CP 2 nerve cells. This excludes the possibility that the CP 2 cells going to the pits are responsible for the discharges on touch. Since they might be chemoreceptors, distilled water, concentrated salt solutions and some organic material were applied to the pits, but no responses in either the CP 2 bundle or the hair fibre bundle were obtained. It therefore remains to be shown whether or not these cells of CP 2 are but part of the normal proprioceptor components of this organ.

From the location of the pits, it might be expected that responses would be obtained from them when they are being covered by the joint membrane. But in preparations in which touch with a brush resulted in responses, extension of the propodite at different rates, failed to do so. It is possible that under natural conditions stimulation takes place under these circumstances, as the membrane will then be more turgid through the blood pressure.

DISCUSSION

Notwithstanding the considerable differences in the structure of the proprioceptive sense organs in different joints and different genera, they all have sense cells perceiving unidirectional movement and cells responsive to position. This strongly indicates that the as yet unknown relations between the dendrites and the elastic fibres of the strand determine the function of the sense cell. For the sense organs as a whole a development to more compactly built organs may have taken place. For instance the PD-organ of the rock lobster with its many sense cells and indistinct elastic strand may represent a more primitive state than the PD-organ of *Carcinus*. Physiologically a similar perfection may be seen in that fibres influenced to a great extent by both movement and position are rare in crabs and frequent in the rock lobster.

In all genera the proprioceptors of the coxal region appear to be built much more precisely than those of the peripheral joints (Alexandrowicz & Whitear, 1957; Alexandrowicz, 1958). On the other hand, the number of sense cells in several of these organs is very small and thus a few units may transmit as detailed information as can be obtained from many of the peripheral organs. A reason for this difference may be seen in the fact that after autotomy the peripheral organs must be rebuilt. It has been observed that in regenerated legs the structure of the PD-organ can be noticeably different and that more fibres with intermediate types of responses are present. Therefore integration of many signals may be used to compensate for inaccuracy in single units.

It appears that the organs can become specialized for certain functions with ageing of the animal. This points to a functional degeneration of part of the sensory cells. How far a similar process may take place for all kinds of cells and explain the observed discrepancy between number of cell bodies and number of functionally reacting units remains to be determined.

The finding of movement sense in all groups of the Crustacea Decapoda Reptantia raises the question whether this sense is present in other Arthropoda. The available evidence strongly indicates this to be so. The responses obtained in the nerve bundles to the proprioceptive organs during the movement of joints in *Limulus* (see Pringle, 1956) and scorpions (Pringle, 1955) most likely originate in movement receptors. These impulses arise from large sense cells located internally, whereas smaller cells give rise to tonic discharges. According to Pringle the latter are homologous to campaniform sensilla of insects, whereas he mentions the possibility that the large cells may be homologous to the chordotonal organs. It may be that the crustacean joint organs are in a state in which these two sense organs are still combined and that the organs of the lobster represent a first stage in their divergence. It should be mentioned that in arachnoids the joint sense organs occur along the outer, upper side of the leg, whereas in Crustacea they are situated on the inner, lower side.

I am grateful to the Director and Staff of the Plymouth Laboratory for their co-operation and for their hospitality, and especially to Dr J. S. Alexandrowicz for the help he has given me in many ways. The experiments were carried out during my tenure of a visiting Professorship at the Department of Zoology, University of Cambridge. I want to thank Prof. Sir James Gray and Staff for their confidence.

SUMMARY

Sensory fibres responding exclusively to unidirectional movement of joints are present in representative species of all four groups of Crustacea Decapoda Reptantia: Palinura, Astacura, Anomura and Brachyura.

They arise from nerve cells located in sense organs in the region of the joints, which contain additional sense cells. The axons of all these cells form bundles in the main trunk from which single unit responses were obtained by preparation of single fibres or small subbundles.

Potentially each organ contains cells sensitive to unidirectional movement for both directions and tonic position fibres for each of the sides of the mid position. Cells with different thresholds are present in each of the four classes.

In certain organs, especially those of older animals, one or two of the four types of sense cells may be lacking.

Sense cells which share some properties of movement and position-sensitive ones are always present, but truly intermediate cells are relatively scarce, more so in higher forms (crabs) than in lower ones (rock lobsters).

The functional significance of some peculiar pits in the joint regions of *Homarus* is studied.

The presence of movement fibres in other Arthropods is discussed and some points relating to the possible homologies of proprioceptive sense organs in the legs of Arthropods are presented.

REFERENCES

Alexandrowicz, J. S., 1958. Further observations on proprioceptors in Crustacea and a hypothesis about their function. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 379–96.

Alexandrowicz, J. S. & Whitear, Mary, 1957. Receptor elements in the coxal region of Decapoda Crustacea. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 603–28.

Barth, G., 1934. Untersuchungen über Myochordotonalorgane bei dekapoden Crustaceen. *Z. wiss. Zool.*, Bd. 145, pp. 576–624.

Pringle, J. W. S., 1955. The function of the lyriform organs of arachnids. *J. exp. Biol.*, Vol. 32, pp. 270–8.

—— 1956. Proprioception in *Limulus*. *J. exp. Biol.*, Vol. 33, pp. 658–67.

Wiersma, C. A. G. & Boettiger, E., 1958. Unidirectional movement fibres from a proprioceptive sense organ of the crab, *Carcinus maenas*. *J. exp. Biol.*, Vol. 35 (in the Press).

J. mar. biol. Ass. U.K. (1959) 38, 153–167
Printed in Great Britain

EXPERIMENTS ON SOME EFFECTS OF CERTAIN ENVIRONMENTAL FACTORS ON *GRACILARIA VERRUCOSA* (HUDSON) PAPENFUSS

By W. Eifion Jones
Marine Biology Station, Menai Bridge, Anglesey

(Text-figs. 1–3)

At an early stage of an autecological study of *Gracilaria verrucosa* (Hudson) Papenfuss it seemed possible that the best growth of the plants occurred in places, such as the Achill Sound and the Church Island Channel in the Menai Straits, where, in the absence of heavy wave action, strong currents flowed over the plants. Experiments were carried out, in the sea, to investigate the effect of currents on the growth rate; the apparatus used and described here would probably be suitable for the investigation of current effects on other sedentary marine organisms.

These experiments also led to some observations on the effects of illumination on the pigmentation and growth rate of the plants which were supplemented by laboratory experiments. High intensity illumination in the laboratory also demonstrated positive phototropism in the plant.

FIELD EXPERIMENTS

Apparatus

The raft shown in Fig. 1 was designed and constructed in timber. Two of the four parallel compartments (*W* and *Y*) were open at the ends, top and bottom. The other two (*X* and *Z*) had pointed bow and stern sections closing them to the current and also perforated wooden bottoms which were shown, by trials, to be necessary to prevent turbulent upwelling when the raft was moored in a rapid current. The raft was moored astern of an anchored barge in the Menai Straits, both being free to swing with the tidal stream so that the raft faced constantly into the current. The tidal current in this part of the Straits reaches about 3 knots. To prevent the entry of floating weed and other debris, the open compartments were protected at both ends and bottom by ½ in. mesh wire netting. Shrimp netting of ⅜ in. mesh was lashed over the top to prevent floating matter being washed into the compartments by waves. The net also had the effect of shading the interior to some extent.

When first launched the raft floated with 10–12 cm freeboard; waterlogging of the wood later reduced this and eventually net floats had to be attached to maintain a freeboard of 5–8 cm. Water entered rapidly through the perforated bottoms of the closed compartments. The accumulation of water-borne silt in these compartments demonstrated the movement of water in and out of them and that stagnation need not be feared.

In each compartment longitudinal battens were arranged to support four 'Tufnol' plastic bars. Two specimens plants were tied to each bar; thin nylon

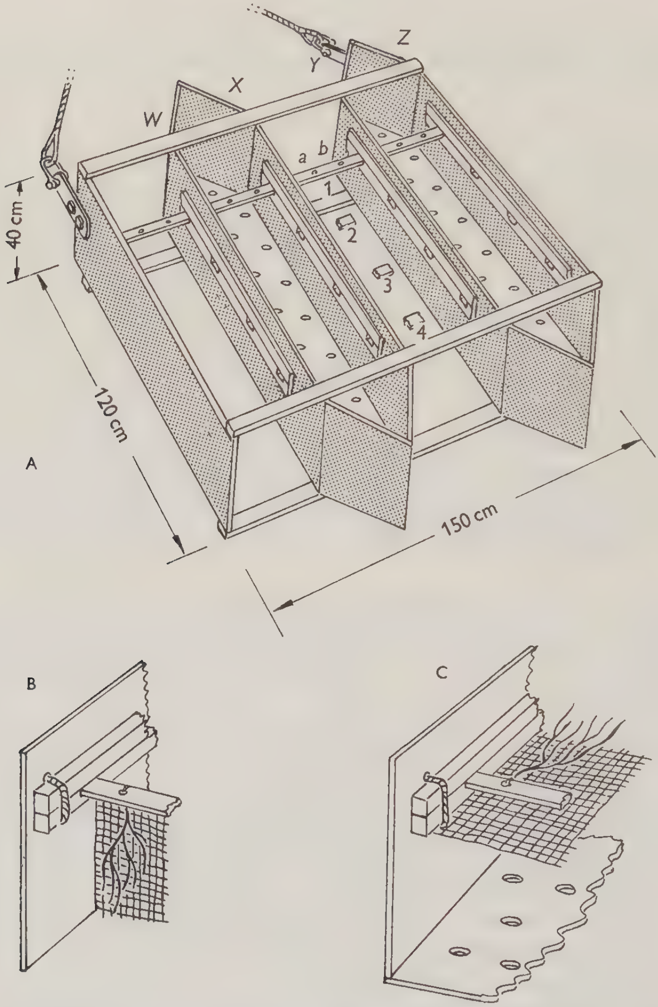


Fig. 1. A, raft used in experiments on the growth of *Gracilaria verrucosa*. Some structural members are omitted for clarity. The four compartments are lettered as shown for reference; the positions of the 'Tufnol' bars bearing the plants are shown in compartment *Y* and were similar in the others. B and C, modifications to compartments *W* and *Z* respectively as described in the text.

fishing gut proved to be the most satisfactory material for this purpose. The bars with the plants attached were removed periodically for examination.

The raft was used in this form during the first season's experiments.

Recording

The specimens used consisted either of a single branched axis with its individual holdfast or else a suitably sized clump of axes arising from part of an expanded holdfast of several seasons' growth. For convenience these specimens are hereafter referred to as 'plants'. The plants were weighed at the start of the experiment and subsequently at intervals of about 10 days. During the first season they were also photographed against a background ruled in 2 cm squares to investigate form changes.

Weighing was carried out with the plants suspended from the arm of a 0–1 g torsion balance so as to be completely submerged in sea water. This method avoided the uncertainty involved if the superficial moisture had to be removed before the plant was weighed in air and also reduced the risk of damage by desiccation during weighing. Although the weight measured was small, owing to the density of the plants being only a little greater than that of sea water, this method was found to be quick and reliable, provided care was taken to ensure that the plants did not touch the sides of the containing vessel or the water surface during weighing and that no air bubbles were trapped amongst the branches.

Results of the first season's experiments

The comparative increases in the fresh weight of a set of thirty-two plants, collected at Criccieth and grown on the raft from 25 June to 6 July 1954, are shown in Table 1. Fig. 1A shows the arrangement of the raft compartments and plant positions. It will be seen that while plants in the open compartments *W* and *Y* increased by about 62%, those in the closed compartments increased by only about 36%. These differences are statistically highly significant. Results in succeeding periods were similar, except where loss of plants or damage occurred.

Conclusions from the first season

It was obvious that growth was more rapid in those compartments open to the current, but it became evident that this was not necessarily due to the direct effect of the current itself. It was observed that the plants in the compartments open to the current lost their original dark red colour, changing to a light straw, while those in the still water compartments were not greatly changed. It is generally accepted that this loss of colour results from strong sunlight and occurs in many red algae in summer. The difference in pigmentation in the current and still water compartments presumably resulted from

TABLE 1. COMPARATIVE INCREASE IN WET WEIGHT OF PLANTS ON RAFT DURING PERIOD 25 JUNE–6 JULY 1954

The values given for the weights are the torsion balance scale deflexions. These are almost exactly one-third of the fresh weight in g.

		Weight			
	Plant	25. vi.	6. vii.	Gain	% gain
W. Open	1*a*	0·57	0·90	0·33	57·89
	b	0·46	0·81	0·35	76·09
	2*a*	0·77	1·51	0·74	96·10
	b	0·27	0·52	0·25	92·59
	3*a*	0·37	0·60	0·23	62·16
	b	0·30	0·03*	—	—
	4*a*	0·96	1·22	0·26	27·08
	b	0·79	0·97	0·18	22·70
		Mean gain 62·09 %			
X. Closed	5*a*	1·91	2·74	0·83	43·45
	b	0·13	0·24	0·11	86·61
	6*a*	2·43	2·86	0·43	17·69
	b	0·26	0·30	0·04	15·38
	7*a*	1·76	2·33	0·57	32·38
	b	0·47	0·68	0·21	44·68
	8*a*	2·28	2·81	0·53	23·45
	b	0·80	1·02	0·22	27·50
		Mean gain 36·14 %			
Y. Open	9*a*	0·50	0·90	0·40	80·00
	b	0·19	0·32	0·13	68·42
	10*a*	1·30	2·12	0·82	63·07
	b	0·25	0·45	0·20	80·00
	11*a*	0·89	1·40	0·51	57·30
	b	0·17	0·30	0·13	76·57
	12*a*	1·88	2·78	0·90	47·87
	b	0·36	0·44	0·08	22·22
		Mean gain 61·93 %			
Z. Closed	13*a*	2·02	2·88	0·86	42·57
	b	0·42	0·48	0·06	14·29
	14*a*	1·20	1·65	0·45	37·50
	b	0·57	0·77	0·20	35·09
	15*a*	0·77	1·02	0·25	32·47
	b	0·29	0·40	0·11	37·93
	16*a*	0·40	0·65	0·25	62·50
	b	0·19	0·25	0·06	31·57
		Mean gain 36·74 %			

Analysis of variance

Source of variation	Degree of freedom	Sum square	Mean square	Variance ratio	Probability
Current	1	5229·83	5229·83	11·3	1·0—0·1 %
Pairs of compartments	2	1·53	0·77	·0017	> 20 %
Replication	27	12532·81	464·17	1·0	> 20 %
Total	30				

* Plant damaged: in calculation this value taken as equal to mean of remaining plants in block I.

differences in illumination. The shading by the partitions caused some reduction in the amount of light reaching the lower parts of each compartment. Thus, where the current was flowing, the plants were trailed out horizontally, level with the tufnol bars, less than 10 cm below the water surface and so received all the light available at that level. In the closed compartments, on the other hand, the plants hung downwards most of the time and therefore received less light because of the shade cast by the partitions and the mutual shadow of their own fronds. Thus the results obtained might be due primarily to differences in illumination rather than conditions of current or still water. In the following season the experiment was redesigned to investigate this.

Modification of the apparatus

One closed compartment (*X*) was left unchanged. In the other (*Z*) a net was arranged horizontally below the tufnol bars so that the plants were held permanently spread level with them (Fig. 1 C). One open compartment (*Y*) was not altered; in the other (*W*) a series of nets was fitted, one behind each bar so as to prevent the plants being swept out horizontally by the current which, instead, kept them pressed against the nets in the hanging position (Fig. 1 B). As the currents flowed for only part of the day (on the flood and ebb) and as some movement of the free plants occurred in all but a flat calm, the new conditions meant that plants in compartment *Z* would obtain the most light and those in *W* the least.

Recording was carried out as before except that the plants were not regularly photographed and that a note was kept of the changes in their colour.

Results

Table 2 gives the results of these experiments. As the periods between successive weighings were not all of equal length, the weight increases are divided by the length of the period and presented as percentage increases in weight per day. The table includes the changes made in the position of the groups of plants after the first growth period (24 May–8 June), when those in the current compartments were exchanged with those in still water, and also shows the observed colour changes in the plants.

During the first growth period the plants in compartment *Z* (held horizontal in still water) and those in *Y* (free in current) showed an average daily increase in weight of 5·33 and 4·39% respectively. Those in compartment *X* (free in still water) and in *W* (held vertically in current) showed, respectively, 2·75 and 2·82% increase per day. Statistical analysis showed that the differences resulting from conditions of still water and current were not significant, but that the difference between the increase in the plants which received most light (in *Z* and *X*) and those which were shaded was highly significant.

TABLE 2. GROWTH OF *GRACILARIA VERRUCOSA* SHOWN AS PERCENTAGE INCREASE IN FRESH WEIGHT PER DAY IN CONDITIONS OF CURRENT AND STILL WATER, FULL LIGHT AND SHADE ON A RAFT DURING 1955

(1) Condition of plants on 24 May	(2) Compartment occupied 24 May–8 June	(3) % gain per day 24 May–8 June	(4) Condition of plants on 8 June	(5) Compartment to which transferred 8 June	(6) % gain per day 8 June–21 June	(7) Condition of plants on 21 June	(8) % gain per day 21 June–5 July	(9) Condition of plants on 5 July	(10) Condition of compartment on 5 July and treatment	(11) % gain per day 5 July–27 July	(12) Conditions of plants on 27 July
Fully pigmented	*Z* (held horizontal in still water)	5·86	All red colour lost	*W*	0·58	Some red colour regained	2·57	Red colour almost fully regained	Many filaments of algae on nets. Cleared 5 July	2·18	Fully pigmented
		1·91*			0·78		3·16			1·64	
		4·91			1·57		0·92			3·95	
		5·80			0·57		0·82			—	
		4·79			1·14		0·09*			—	
		4·86			—		3·18†			4·54	
		5·15			1·26		1·46			0·24*	
		6·20			1·21		1·56			—	
	Mean	5·33			1·01		2·02			3·08	
Fully pigmented	*Y* (free in current)	5·00	Red colour much reduced	*X*	3·00	Most red colour lost	2·10	Most red colour lost		—	Most red colour lost
		4·42			4·37		2·36			3·54	
		—			3·54†		2·35			—	
		—			4·95†		4·13			2·97	
		—			5·45†		3·31			2·98	
		—			4·00†		1·93			3·26	
		4·85			2·31		0·74			3·72	
		4·29			3·42		2·40			—	
	Mean	4·39			3·92		2·41			3·29	
Fully pigmented	*X* (free in still water)	3·85	Red colour slightly reduced	*Y*	2·97	Red colour greatly reduced	1·86	Red colour largely restored	Heavy growth of filamentous algae. Cleared 5 July	2·86	Red colour much reduced
		1·35			4·28		—			4·86	
		1·67			2·35		2·33			—	
		—			3·48		3·28			—	
		3·84			2·15		2·72			6·18	
		4·34			4·85		0·82			1·69	
		1·47			2·53		2·71			6·05	
		2·64			4·96		1·86			4·77	
	Mean	2·75			3·42		2·23			4·40	
Fully pigmented	*W* (held vertical in current)	4·10	Colour unaffected	*Z*	3·15	All red colour lost	2·55	All red colour lost		4·14	All red colour lost
		3·55			4·77		2·46			1·46	
		1·69			5·02		1·93			0·24*	
		1·33			4·58		2·26			2·82	
		2·56			5·04		2·29			2·52	
		3·66			4·86		2·85			2·55	
		0·46*			2·30		1·63			—	
		—			2·76†		4·86			1·82	
	Mean	2·82			4·06		2·60			2·71	

Notes. Each horizontal row concerns the same plants throughout the experiment.

* Damaged plants: not included in calculations.

† New plants introduced to replace casualties.

Conclusions

It appears from these results that the effect observed in the earlier experiment was only secondarily due to current and resulted from the better illumination received by the plants spread out by the flow of water. Later experiments were made in the laboratory on the direct effect of current.

The plants in compartment *Z*, being constantly in a horizontal position, probably received more light than those in *Y*, which were horizontal only when the tidal currents flowed. The growth rate in *Z* did appear, from its mean value of 5·33%, to be higher than that in *Y* (4·39%), but statistical analysis did not show the difference to be significant.

Causey, Prytherch, McCaskill, Humm & Wolf (1946) showed that unshaded *Gracilaria* reached its maximum growth rate at 60 cm below the surface without improvement at lesser depths. In the present experiment pronounced differences in growth occurred in plants within the upper 35 cm of water but, owing to shading, plants nearer the surface received very much more light than the lower ones. Measurement of the light reaching the plants with the raft afloat and the nets in position was not practicable. A Weston meter showed that when the raft was out of the water the shading effect of its sides reduced illumination at the bottom of the compartments to about one-eighth of that at the level of the tufnol bars. Thus, if plants at the latter level were receiving 25% of the incident light owing to loss at the water surface (Sverdrup, Johnson & Fleming, 1942), then, after allowing for the difference shown by the meter, the lower parts might receive about 3%.

Bleaching effects

During the first growth period (24 May–8 June) the plants in the two well-illuminated compartments changed colour from their original dark red to a light straw colour as the photolabile phycoerythrin was lost. The colour of the plants in the two shaded compartments was virtually unaltered.

After weighing at the end of the period (on 8 June) the plants were replaced in different compartments for the remainder of the experiment. These changes are shown in Table 2. They involved interchange of the plants from compartment *W* (current and shade) with those from *Z* (still water and good illumination) and a similar interchange between compartments *X* (plants hanging in still water) and *Y* (plants free in current).

In the next period (8–21 June) bleached plants in the 'shaded' compartment *W* showed a much lower growth rate than unbleached plants in the same compartment in the previous period, while the unbleached plants in the 'light' compartment *Z* showed a high rate comparable to that of the first period. During this and subsequent periods the plants in *W* regained their colour while, at the same time, their growth rate increased, the successive average values for the daily rise in fresh weight being 1·01, 2·02 and 3·08%.

The latter value was rather higher than that for unbleached plants in the same compartment during the first growth period but may have been erroneously high as damage and loss had reduced the numbers of plants in the compartment and adversely affected the replication. At the same time, in compartment *Z*, the plants became bleached while growing rapidly (4·06% per day) in the 8–21 June period and, in the subsequent periods, grew at lower rates of 2·60% and 2·71% per day. This rate was similar, incidentally, to that of fully pigmented plants in the shaded compartments.

Meanwhile, settlement of the raft as its timbers became waterlogged and the loss of the attached floats frequently resulted in its being virtually awash and this, while not greatly affecting plants in *W* and *Z*, greatly reduced the shading effect in compartment *X* owing to the plants in it being swayed by movements of the raft and the shipping of water. In fact a reverse shading effect occurred between compartments *X* and *Y* owing to the rapid growth of filamentous algae in the latter (open to current) which shaded the *Gracilaria* plants and slowed their growth. At the same time, the plants in *Y* regained their colour and when, on 5 July, the shading weed was removed, a period of rapid growth followed comparable to that during the first period (24 May–8 June) and in both cases resulting, presumably, from the full illumination of unbleached plants.

Before considering the final conclusions to be drawn from these results it is proposed to describe supplementary experiments carried out in the laboratory.

LABORATORY EXPERIMENTS

EXPERIMENTS ON THE EFFECTS OF WATER CURRENTS ON GROWTH RATE

As a check on the current/still water experiment plants were grown in 3 cm. diameter Pyrex glass tubes. Four tubes were used and supplied with sea water from the laboratory system via a constant head device. The outflow from each tube was controlled by means of a screw clip closing a rubber tube. The rate of flow from each tube was found by timing the collection of a measured volume from the outlet. To prevent any rise in temperature the tubes rested in a tray and tap water was directed on to each. A thermometer was placed in each tube and showed that no temperature differences occurred.

Weighed portions of a single plant, divided at its expanded basal holdfast, were placed in the tubes. They were grown for 9 days under constant, even illumination of 600 lux from daylight type fluorescent tubes and then weighed again.

Results

Table 3 gives the results obtained.

TABLE 3

Approx. rate of flow (ml./h)	Increase in fresh weight (%)
22	7·7
1,500	14·9
7,200	20·4
36,000	20·2

Conclusions

It appears that the growth rate increases with increasing flow up to about 7 l./h, after which, under the conditions of light and temperature prevailing here, further increase in the flow rate does not induce faster growth. These results must, of course, be treated with great caution owing to the lack of replication and the shortcomings of the apparatus, in which it was difficult to maintain a steady flow. However, a flow rate of 7 l./h is equivalent to a current of about 1 m/h through the tube, so that it seems that currents cannot be expected to have a direct effect on the growth rate in the field, even allowing for the higher light intensity which the plants would receive for part of the time under natural conditions. More movement of water would occur even in the calmest conditions and, in fact, it seems that current becomes a limiting factor only when the water moves so slowly as to be almost stagnant. This is in agreement with the observations of Gessner (1940) who showed that respiration and assimilation rates in *Fucus* spp. were retarded up to 50% in stagnant water, compared with water in a shaken vessel.

EXPERIMENTS ON EFFECTS OF HIGH INTENSITIES OF ILLUMINATION

The bleaching effect observed on the shore and in the raft experiments occurred under conditions of intense summer illumination. Confirmatory experiments in the laboratory therefore require the provision of light of the same order of intensity.

Apparatus

In these experiments, two Strand Electric pattern 23 N theatre spotlights were used. These employ 500 W projector bulbs in highly efficient spherical reflectors and have a lens system concentrating the light in an 11° cone. They were set up so as to shine on to a mirror, inclined at 45° to the horizontal, which deflected their beams downwards. (This was to allow the lamp filaments to burn in the correct vertical position.) At a total distance of 65 cm from the front lenses the light from both lamps coincided on an area about 20 cm in diameter and here there was placed a white enamel dish 5 cm deep, through which a constant stream of sea water was passed, without causing ripples on the surface. Although the lamps produced considerable heat it was found possible to prevent the temperature of the water in the dish rising above its original level (that of the laboratory supply) by suitably adjusting the rate of flow.

The light intensity was not uniform over the whole of the illuminated area; in the brightest, central area the intensity of illumination was about 56,000 lux, as measured by a Weston meter with Invercone calibrated from a standard source. This value fell off away from the centre of the illuminated area and was of the order of 30,000–35,000 lux at the periphery. It must be borne in mind that the spectrum of the light from tungsten filaments is very different from normal daylight. The former is deficient in the ultraviolet and the shorter wavelengths of the visible spectrum. Thus, although in these experiments the measured intensities are comparable and the results similar, no quantitative correspondence should necessarily be expected between the results of the laboratory and field experiments.

Methods

Both mature plants and sporelings of *Gracilaria verrucosa* have been used. With the former it was intended to test the effect of increasing daily periods of intense illumination and at the same time to observe whether any phototropic response occurred. The latter phenomenon seemed likely from the observation of some forms assumed by the plant in the field. To prevent confusion of the results by the reflexion of the light upwards from the bottom of the dish and to hold the plants in a fixed position, they were attached by nylon thread to perforated 'Tufnol' plastic plates, each about 23 × 7 cm. so that no branches projected beyond the edges. When not under the bright light, the plants received constant illumination of about 500 lux from fluorescent lamps. In the first experiment three 'plants' were used, all parts of the same tetrasporic clump. The fresh weight of each was recorded before the experiment began and their colour checked against an arbitrary standard designed to show the change from complete bleaching to full pigmentation in eight numbered steps.

One of the plants was left in the dish under a constant illumination of 500 lux all the time to serve as a control, the others were given additional daily periods under the bright lights of 30 min and 1 h respectively for the first run of 12 days, these periods being increased in subsequent runs.

As only two plants could be accommodated under the lamps at once and as daily periods of 8 h were envisaged in the later stages, replication of this experiment was not practicable and this must detract from the reliability of the results.

Phototropism

At the end of the first run of 12 days the form of the control plant was unchanged. The branches of the other two, which had received respectively 6 and 12 h of strong illumination, were turned upwards towards the light

(Fig. 2), except where they were secured to the tufnol plates. After each weighing between runs these plants were turned over before being tied again to the plate; in each case the upward curvature was quickly re-established. Although appreciable growth of the control took place during the experiment, no upturning of its branches occurred. It may therefore be concluded: (i) The upturning is not due to gravity, since this factor affected all plants, including the control, equally. (ii) There is a pronounced positive phototropism in response to bright light of the order of full daylight but not, apparently, in response to lower intensities. As the lower intensities are sufficient to promote growth, it seems possible that the upturning is not so much the result of an auxin migration (its usual explanation in higher plants), which might be expected to occur under all intensities, but is a direct effect of the high light intensity, perhaps by speeding up growth of the more shaded and therefore less bleached lower side (see below). (iii) The response has been observed after as little as 3 h total intense illumination.

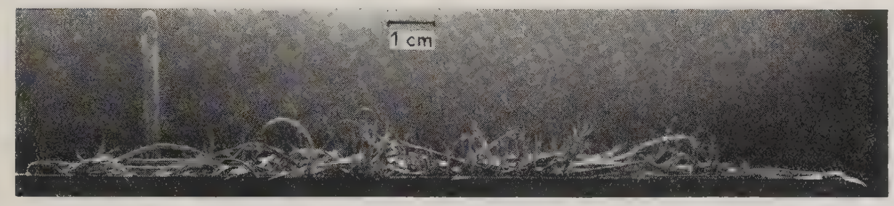


Fig. 2. Plant of *Gracilaria verrucosa* showing response to unidirectional illumination. Those branches which are not secured to the 'Tufnol' plate are turned upwards towards the incident light. The hooking over of the extreme tips of the branches was caused by desiccation during their photography. The scale line is 1 cm.

Changes in pigmentation and growth

The main changes observed and the length of the daily high light period in successive runs are shown in Table 4.

Pigmentation changes became noticeable during the second run, the parts of the plants under the brightest area of the field becoming lighter in colour, while the remainder in the less intense light lost less of their pigments. It was also noticed that the loss of pigment was, at this stage, restricted to the sides of the fronds facing the light, while the sides in contact with the tufnol plates were less affected. As the daily light period was increased, the bleaching effect became more marked until almost all the plant was bleached in each case. Little change in pigmentation occurred in the control. The temperature in both dishes throughout the experiment was maintained between 12·5° and 14·5° C.

During one run the control plant was placed on the white bottom of the dish and not on the tufnol plate. During this period a fourfold increase in the growth rate occurred in the control compared with that in other periods, which may be attributed to the increased light obtained by reflexion upwards from the bottom of the dish and internal reflexion between this and the water surface. Normally most of the light passing the plant would be absorbed by the tufnol plate.

The effect of increasing daily illumination is shown in Fig. 3, which also indicates the state of pigmentation. It will be seen that there appears to be a direct relationship between the daily illumination and the increase in fresh weight as long as pigmentation is not greatly affected, but that after bleaching occurs the growth rate, so far as can be judged from the data available, does not rise very much with further increases in the period of illumination and falls

TABLE 4. GROWTH OF *GRACILARIA VERRUCOSA* WHEN SUBJECTED TO HIGH-INTENSITY ILLUMINATION

Duration of run...				17–29 May			29 May–9 June			9–18 June			18–29 June			29 June–9 July		
Plant...	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Daily hours of bright light	—	—	—	0	0·5	1·0	0	2·0	3·0	0	5·0	4·0	0	6·0	2·5	0	8·0	8·
% gain in fresh weight at end of run*	—	—	—	2·0	6·5	10·1	31·4†	20·1	29·0	7·7	25·9	23·6	8·8	33·3	15·5	3·0	19·8	20·3
	(17 May)																	
Colour at‡ end of run	6	6	6	6	6	6	6	3–5	2–5	6	1–4	2–4	5–6	1–2	2–4	5–6	1–2	1–2

* Corrected to give equivalent for 12 days.

† Plant '*a*' not on tufnol plate during run 29 May–9 June—received better illumination than in other runs.

‡ Values given are according to arbitrary standard where 1 = bleached, 8 = very dark. Where two values are given the lower figure represents the colour of the parts of the plant under the brightest portion of the field and the higher one the remaining darker parts.

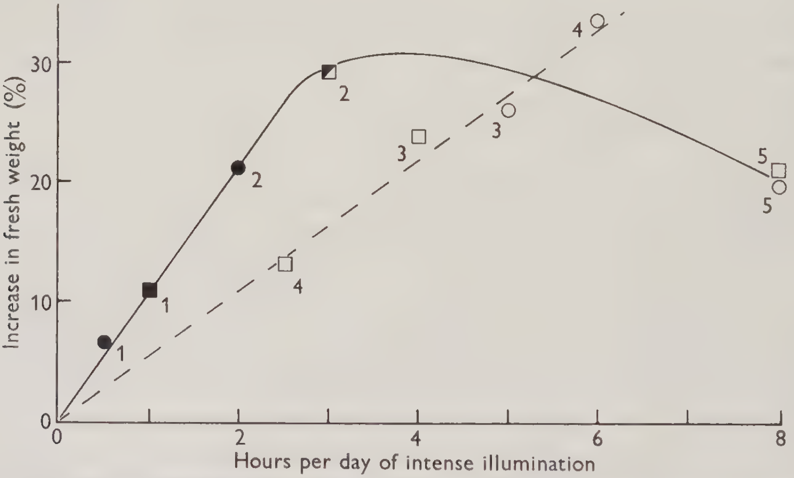


Fig. 3. The effect of increasing daily dosage of intense illumination on the growth and pigmentation of *Gracilaria verrucosa*. The numerals alongside each symbol indicate the consecutive periods of illumination. The vertical scale represents percentage increase in fresh weight in 12 days. Symbols: ●, 1st plant; ■, 2nd plant. Pigmentation: ■, fully pigmented; ◩, half bleached; □, fully bleached; — — —, growth in fully bleached condition.

appreciably when the daily dosage is raised to 8 h. The growth of plant '*c*' during the fourth run (18–29 June), when in the bleached condition, was only about half that obtainable in an equal period of illumination when unbleached. In fact the values of the percentage increases in weight of the plants in the bleached state, for periods of illumination up to 6 h, fall on a straight line whose slope is approximately half (actually 1:1·9) that for the unbleached plants, a value which agrees with the results of the raft experiments.

When the same lighting system was used in experiments on sporelings the results were very different. Sporelings at an early stage of development, up to 1 week old, grown from carpospores shed in the laboratory, were illuminated by the light for periods of up to 1·5 h daily. Although there was a slight initial increase in growth rate, the loss of phycoerythrin was very rapid and development ceased after a total of 4–5 h illumination. The sporelings did not recover from this treatment. Controls which received constant illumination of 400 lux grew normally and retained their colour.

GENERAL CONCLUSIONS AND DISCUSSION

The following conclusions may be drawn from the results of the experiments described here, bearing in mind that additional, more refined, experiments would be required to place them on a more exact quantitative basis.

(i) Phycoerythrin in living *Gracilaria verrucosa* disappears under the action of light intensities of the order of full daylight.

(ii) When the light is reduced, phycoerythrin is regenerated. In shade where the light intensity is approximately 12% of that just below the sea's surface, considerable regeneration occurs in ten days.

(iii) The bleaching effect does not appear to depend on unusually high temperature. In the laboratory it occurred at under 14·5° C and in the field the temperature was substantially the same (rising from 10° to 14° C), while both regeneration of pigments and their bleaching proceeded.

(iv) The growth rate of the plant, under all lighthing conditions so far observed, is lower in the bleached condition than when fully pigmented. At the low intensities under which phycoerythrin is regenerated unbleached plants grow from twice to three times as fast as bleached ones; at intensities high enough to cause bleaching, unbleached plants grow from 1·5 times to twice as fast as bleached plants.

The slower growth of bleached plants points to phycoerythrin being directly useful to the plant in its growth processes. This reinforces, in terms of the growth rate, what has been demonstrated by several workers in the measurement of assimilation rates in the Rhodophyceae. Wurmser & Ducleaux (1921) showed that the assimilation rates of *Rhodymenia* and *Chondrus* when red were twice as great as when green (i.e. bleached, presumably), a value which fits in well with the present results.

Although the loss of phycoerythrin reduces the growth rate of the plant this does not appear to be a serious difficulty in nature since bleaching is caused by the high summer illumination, and at these intensities the growth, even of bleached plants, is rapid. On open shores with clear water bleaching begins to be noticeable in late spring and early summer and it is at this time, when the light intensity is high but before the plants lose all their phycoerythrin, that growth is fastest. Later bleaching becomes more pronounced and the growth rate falls but the maximum size is reached in late summer when the plants are fully bleached.

The reduction in the growth rate of plants which have been intensely illuminated for a long daily period is also in agreement with previous work: Meyers & Burr (1940) showed that, in *Chlorella*, high light intensities (above 15,000 lux) reduced the rate of photosynthesis. The plants recovered from this high illumination, recovery being slower the longer the period of illumination and the higher the intensity, but at very high intensities permanent injury took place. In the case of sporeling material of *Gracilaria*, injury from which the sporelings do not recover occurs under intensities of the order of full daylight when this is received for 4–5 h. This can explain why plants of *Gracilaria* are not found in upshore pools where intensities of this order may be received for considerable periods every day in summer.

This work was carried out at the suggestion of Prof. L. Newton, whose encouragement and helpful comments I should like to acknowledge.

SUMMARY

A raft is described which permits experiments on the effect of current and still water on marine organisms. This has been used in experiments on *Gracilaria verrucosa* which, supplemented by laboratory experiments, show that, while there is no direct current effect on the growth, there is an indirect effect. This is an increase in the growth rate due to the better illumination of plants spread out by the current.

The bleaching of the thalli when illuminated by light of high intensity has been observed on the raft and demonstrated under tungsten spotlamps in the laboratory. It has been shown that the colour is regained when the light is reduced and that growth is from one and a half to three times as fast in unbleached plants as in those from which the phycoerythrin has gone. This difference between bleached and unbleached plants is less marked at high light intensities.

Positive phototropism of the branches of the plant has been demonstrated.

High light intensities have been found to have an adverse effect on mature plants only after prolonged illumination but to be quickly harmful to sporelings. It is suggested that this may limit the upshore distribution in pools.

REFERENCES

CAUSEY, N. B., PRYTHERCH, J. P., MCCASKILL, J., HUMM, H. J. & WOLF, F. A., 1946. Influence of environmental factors upon the growth of *Gracilaria confervoides*. *Bull. Duke Univ. Mar. Sta.* No. 3, pp. 19–24.

GESSNER, F., 1940. Die Bedeutung der Wasserbewebung für die Atmung und Assimilation der Meersalgen. *Jb. wiss. Bot.*, Bd. 89, pp. 1–12.

MEYERS, J. & BURR, G. O., 1940 Studies on Photosynthesis: some effects of high light intensities on *Chlorella*. *J. gen. Physiol.*, Vol. 24, pp. 45–67.

SVERDRUP, H. V., JOHNSON, M. W. & FLEMING, R. H., 1942. *The Oceans, their Physics, Chemistry and General Biology*. New York.

WURMSER, R. & DUCLEAUX, J., 1921. Sur la photosynthèse chez les Algues Floridées. *C.R. Acad. Sci., Paris*, T. 171, pp. 1231–1233.

J. mar. biol. Ass. U.K. (1959) 38, 169–188
Printed in Great Britain

STUDIES ON MARINE FLAGELLATES

V. MORPHOLOGY AND MICROANATOMY OF *CHRYSOCHROMULINA STROBILUS* SP.NOV.

By Mary Parke
The Plymouth Laboratory

Irene Manton and B. Clarke
Botany Department, Leeds University

(With total of 44 Figures in text and on Plates I–VIII)

CONTENTS

PAGE
Introduction 169
Formal taxonomic diagnosis 170
  Chrysochromulina strobilus sp.nov. (Plymouth no. 43) 170
Observations with the Light Microscope 172
  Description and behaviour of the living cell 172
  Contents of the body 175
  Reproduction 177
Observations with the Electron Microscope—morphology 177
Observations with the Electron Microscope—anatomy 178
  Haptonema 178
  Scales 181
  Internal Organs 181
  Pyrenoid 182
  Flagellar Bases. 182
  Golgi 182
  Muciferous Bodies 183
Discussion 183
Summary 186
References 186
Appendix. Recorded distribution of *Chrysochromulina strobilus* sp.nov. 187
  Seasonal distribution of *C. strobilus* sp.nov. 188

INTRODUCTION

The new species to be described here is very common in the English Channel, though like other members of this genus it is so fragile that it needs to be cultured to be effectively detected. This particular species has been under observation for some years, but publication has been deferred until sections of it could be made available. The observations involved in the taxonomic description have been based on two early isolates numbered 4 and 43 in the Plymouth collection. More recently, however, it has been encountered frequently in routine sea-water samples brought into temporary culture to record the nanoplankton forms present, and we are therefore able for the first time to give tables of seasonal and depth distributions for the incidence of the species, at various stations. In Table 1 of Appendix (p. 187) its occurrences are listed, all being from water-bottle samples, except those taken on 23 August 1950 and 6 November 1957 (fine tow-net) and on 19 June 1957 (very fine tow-net). Table 2 (p. 188) gives densities at different depths sampled on one day at Hydrographic Station E 1.

The other technical methods involved are substantially as before except for minor variants in fixing and embedding procedures. As on previous occasions the electron micrographs of external morphology were carried out on the old Philips microscope in the Leeds Botany Department. A few of the high-power details, notably those on haptonema structure contained in Pl. VIII, were obtained on the Siemens Elmiskop I at the Rockefeller Institute which was made available during a 2-week visit at Christmas 1957. The remaining anatomical micrographs have been taken on the new Siemens Elmiskop I recently installed in the Leeds Botany Department by means of a grant from the Rockefeller Foundation.

Very grateful thanks are due to Dr K. R. Porter of the Rockefeller Institute for his unfailing courtesy and help during flying visits to his laboratory, also to the Rockefeller Foundation for removing the necessity for such visits. For help with the Latin diagnosis we have to thank Dr T. Christensen of Copenhagen. We have also to thank Miss I. Adams for assistance in the routine examination of samples, Mr F. G. C. Ryder for designing and building the special apparatus used for the growth of the very numerous temporary cultures from which distribution records have been obtained, Miss D. Ballantine (Mrs B. Hepper) for testing this organism for its possible toxicity to fish, Dr L. H. N. Cooper, Mr E. I. Butler, Dr T. J. Hart ('Discovery II'), Mr D. Vaux and Mr A. C. Burd ('Sir Lancelot') for the collection of sea-water samples.

FORMAL TAXONOMIC DIAGNOSIS

Chrysochromulina strobilus sp.nov. Parke & Manton

(Gr. *στρόβιλος*—a pirouette)

Motile cells showing considerable metaboly; dorsi-ventrally flattened, convex on the dorsal surface, flat or concave ventrally; when stationary or gliding slowly body saddle-shaped or appearing truncate-ovate in dorsal or ventral view; when swimming rapidly bell-shaped, obovoid or depressed-globose; 6–10 (exceptionally 5–12) μ in size. Two flagella and one haptonema arising fairly close together from the ventral surface, usually one-third cell length from rounded end in a centre line; flagella subequal to equal, very fine, smooth, tapered to a small knob (E.M. observation), appearing homodynamic when cell moving rapidly and heterodynamic when cell moving slowly or stationary, 2–3 times body length; the haptonema capable of attaching along its whole length, half the thickness of the flagella, 12–18 (exceptionally 20) times the body length when fully extended, with a swollen tip and an internal structure of three concentric membranes surrounding a ring of six 'fibres'. The periplast, pectic in nature, showing a surface pattern of tightly packed angular 'cup' scales 0·15–0·2 μ in diameter; additional very thin, transparent, circular to oval, sculptured scales, 0·3–0·4 μ in diameter, with a pattern of radiating ridges, present beneath the 'cup' scales.

Cells uninucleate, no stigma. Chromatophores faintly striated on outer face, 2 or 4, occasionally 1 or none, golden brown; in cells of motile phase parietal, saucer-shaped to oblong, lacking an external pyrenoid but with a well-marked internal storage region; in non-motile phase pale gold and very finely lobed. Lipids and leucosin produced. Ejectile muciferous bodies small, distributed in peripheral cytoplasm, more numerous on dorsal and ventral surface of back of saddle but their position changing with the metaboly of the body. Nutrition phototrophic and/or phagotrophic. Non-toxic to fish.

In motile phase asexual reproduction by fission into two daughter-cells, usually of equal size; in non-motile phase by successive fission of amoeboid cells to produce 4 ovate daughter-cells with thin walls; motile phase liberated from walled daughter-cell through pore.

Habitat. The sea at position lat. N. 49° 21′, long. W. 04° 54′ (9 May 1950, type culture) at surface. Type culture (Plymouth no. 43) deposited with the Culture Collection of Algae and Protozoa, Cambridge.

Cellula in statu erratico satis metabola, depressa, dorso convexo, ventre plano vel cavo; dum quieta lenteve prolabens ephippioides seu a dorso vel ventre truncato-ovata visa; dum cito natans campanuliformis seu obovata seu depresse globularis; 6–10 (raro 5–12) μ longa. Flagella duo haptonemaque unicum in facie ventrali sat conferte inserta, plerumque mediana, tertia cellulae longitudinis parte ab apice rotundato remota; flagella paene vel plane aequalia, tenuissima, glabra, ad apices attenuata, nodulo quidque terminatum (per microscopium electronicum viso), cellula 2–3 plo longiora, inter motum citum homodynamica, inter lente movendum ut inter quietem heterodynamica visa; haptonema flagellis dimidio tenuius, extensum cellula 12–18 (raro –20) plo longius, apice incrassatum, in sectione transversa tres membranas tubiformes concentricas ostendens fibras 6 in orbem dispositas induentes, in tota longitudine adhaerendi potens. Periplastum pecticum, squamis dense angulate congestis, 0·15–0·2 μ diametro, marginibus adscendentibus, discis intus mucronato-incrassatis obtectum, alteris illis suppositis delicatulis, hyalinis, orbicularibus vel ovalibus, 0·3–0·4 μ diametro, costis radiantibus ornatis.

Nucleus unicus; stigma nullum. Chromatophora 2 vel 4, interdum unum vel nullum, fulva, in facie externa striatula, inter statum erraticum cellulae parietalia, catilliformia vel oblonga, pyrenoidibus externis carentia, sed regione penaria interna manifesta quidque instructum; inter statum sedentarium pallide aurea, subtilissime lobata. Synthemata lipoida et leucosinea. Corpora mucifera ejectilia parva, in strato externo cytoplasmatis distributa, in facie dorsali et ventrali posterioris partis ephippii crebriora, inter metabolam situs mutantia.

Alga et phototropha et phagotropha seu alterutro solum victu alta; piscibus non venenosa.

Propagatio vegetativa in statu erratico bifissione effecta, cellulis filialibus plerumque aequalibus; in statu sedentario fissione iterata cellulae amoeboidis, cellulis filialibus 4, ovatis, parietibus subtilibus indutis, quaque earum cellulam erraticam per porum liberante.

Typus die 9. Maji 1950 in summo mari lat. bor. 49° 21′, long. occ. 04° 54′ lectus, in Plymouth Angliae sub numero 43 cultus, postea in vivario Cantabrigiensi depositus.

OBSERVATIONS WITH THE LIGHT MICROSCOPE

Description and behaviour of the living cell

Chrysochromulina strobilus (Figs. 1–12) is somewhat similar to *C. ephippium* and *C. alifera* (Parke, Manton & Clarke, 1956) in shape, size and in the behaviour of the flagella and haptonema when an individual is stationary or when slowly gliding; it also exhibits phagotrophism in common with all our species so far described.

It differs from *C. ephippium* and *C. alifera* in the position of origin of the flagella and haptonema (Fig. 8), the body attitude and the behaviour of the

---

Legends to Text-figs. 1–12

Chrysochromulina strobilus sp.nov.

(Figs. 1–7, × 1250; Figs. 8–12, × 5000)

Fig. 1. Cell anchored by fully extended haptonema which is bent and attaching only by swollen tip.

Fig. 2. Anchored saddle-shaped cell with flagella showing heterodynamic movement; haptonema attached along most of its length.

Fig. 3. Saddle-shaped cell gliding slowly with haptonema lying away from and in front of body.

Fig. 4. Early fission stage rotating slowly and moving with haptonema fully extended in front of body.

Fig. 5. Fission stage with four flagella and two haptonemata in attitude adopted for fairly rapid swimming.

Fig. 6. Individual swimming with flagella in the position characteristic for the species for fairly rapid movement; haptonema extended but coiled up on itself and trailing behind body.

Fig. 7. Individual gliding without rotating with flagella in position characteristic for the species during gliding movement.

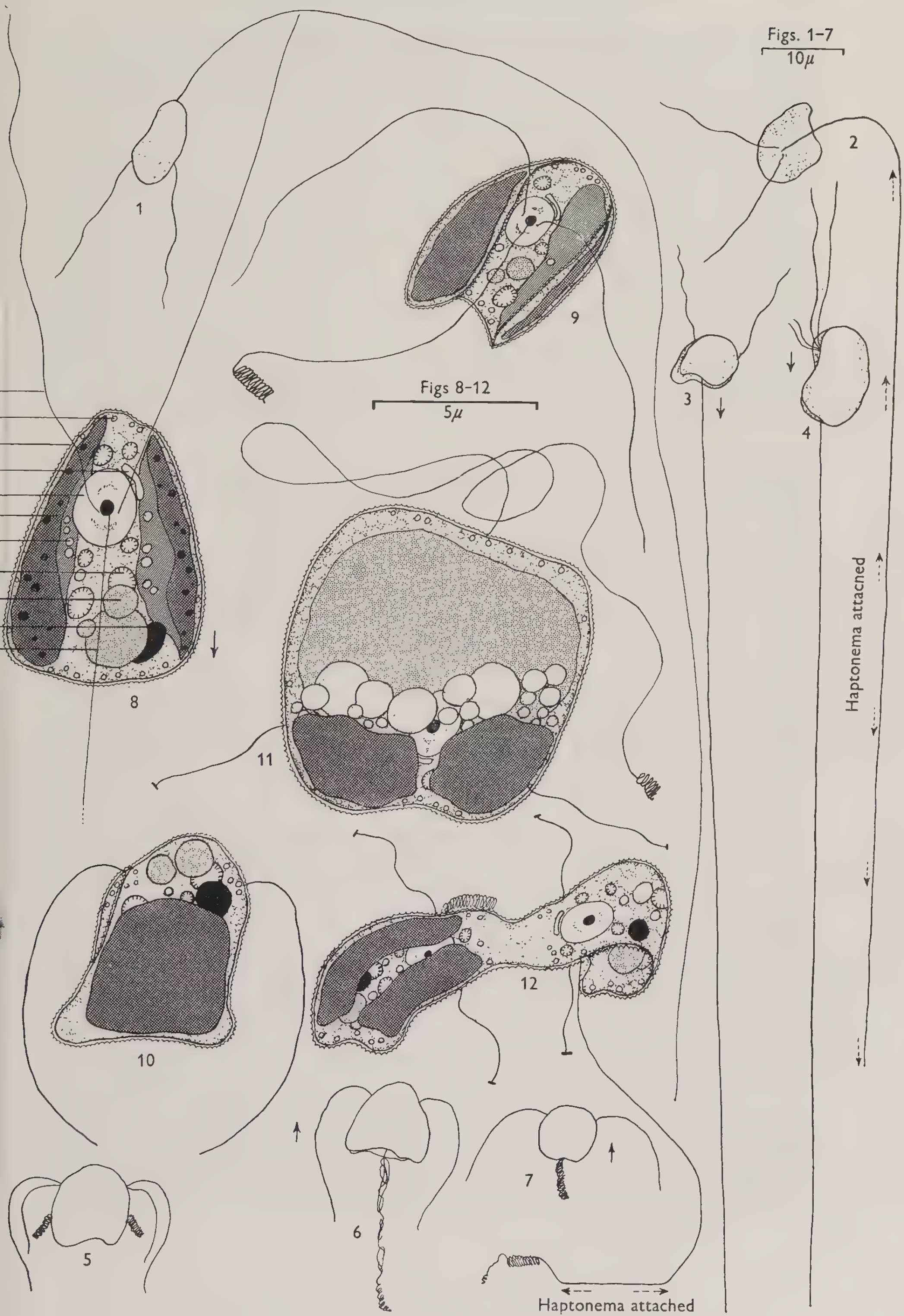
Fig. 8. Kite-shaped cell (ventral surface) moving slowly with haptonema fully extended in front of body, one flagellum slowly undulating, the other stiff or gently vibrating. *c*, chromatophore containing saturated lipid globules stained by Sudan Black; *f*, flagellum; *fb*, lipid globules; *g*, graphite; *go*, golgi; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *mt*, mitochondrion; *n*, nucleus; *s*, 'cup'-shaped scale.

Fig. 9. Anchored cell with haptonema partly coiled and flagella lying out from body.

Fig. 10. Individual swimming with flagella and body in the position characteristic for the species during very rapid swimming.

Fig. 11. Cell containing numerous lipid globules and a large leucosin vesicle from a culture grown in strong light for 10 days; haptonema nearly fully extended, coiled on itself and attached only at tip.

Fig. 12. Late fission stage, one daughter-cell without chromatophores.


Figs. 1–7
10μ
Figs 8–12
5μ
Haptonema attacned
Haptonema attached

Figs. 1–12

flagella during rapid swimming (Fig. 10), the length and prehensile powers of the haptonema (Figs. 1, 2, 12; Pl. I), the absence of a recognizable pyrenoid on the inner face of each chromatophore (Fig. 8), the excessive production of lipid containing bodies (Fig. 11) and in possessing a distinctly patterned, clearly discernible, periplast (Pl. II).

In *C. strobilus* the subequal to equal flagella, arising about 1 μ apart, are extremely fine, and since the species also exhibits a very rapid swimming movement their behaviour is difficult to follow even under dark field. It appears almost certain, however, that when a cell of this species is swimming rapidly the dorsal surface of the saddle is directed forward and the two wings are backwardly directed and incurved (Fig. 10); the cell then appears bell-shaped, if the wings are only slightly incurved, or ovoid to sphaeroidal when the wings are strongly incurved (Fig. 7). The flagella lie round the body and appear to behave homodynamically (Fig. 10; Fig. 22, Pl. II). During rapid swimming the cell moves in straight lines for quite long distances, rotating very quickly with little gyration, but when speed decreases it rotates more slowly showing pronounced gyration with the flagella still behaving homodynamically and being lifted outwards to lie further away from the body (Figs. 5, 6).

When the flagella are raised still farther away from the body the cell shows a gliding movement without rotation (Fig. 7). After a short time under either light or dark field the cells become stationary, each attaching by means of its haptonema which can show any degree of uncoiling from tightly coiled to fully extended (Figs. 1, 2, 9, 11 and 12); the body with the flagella lying away from it then starts to rotate very rapidly to give the appearance, particularly under dark field, of a lighted rotating Catherine Wheel firework; this behaviour can continue for long periods and is extremely characteristic for this species, hence the name *C. strobilus*.

As in *C. ephippium* (cf. 1956, p. 405) the flagella appear heterodynamic when the cells are stationary, gliding slowly, or rotating slowly with haptonema fully extended (Figs. 2, 8). The haptonema itself can either lie across the body (Figs. 1, 2, 9) or out at right angles from it (Fig. 3). It is believed that when the flagella are unequal in length it is the shorter flagellum that remains stiff when one is stationary and the other undulates. In *C. strobilus* the haptonema, arising between the two flagella (Figs. 8, 9), is usually tightly coiled (3 × 1 μ) and not visible during very rapid swimming (Fig. 10) but it can trail behind the body, partly to fully extended, or frequently appearing irregularly tangled (Figs. 6, 7), when a cell is still moving fairly rapidly. Usually, however, when the haptonema is trailing behind the body, the cell moves more slowly and it also shows a more pronounced gyration. The haptonema in this species differs from those of species already described in being capable of attaching itself to a surface along part of its length or along its whole length not merely by means of its swollen tip (Figs. 2, 12); for further comment see

p. 177. In this species also it took a great deal of time and patience to obtain measurements of 100 fully extended haptonemata since a cell with a fully extended haptonema when brought into the light or dark field for measurement will immediately retract its haptonema and swim away. Although the species lacks an obvious stigma it shows a definite phototactic reaction.

In an actively growing culture 65% of the cells are between 6 and 9 μ in size and a culture at the peak of growth contains up to ½ million cells per ml. Occasionally cells lacking chromatophores are seen in the stock cultures (Fig. 12), but in cultures treated with penicillin and streptomycin the colourless cells are not uncommon; they are usually smaller (4–6 μ) than those possessing chromatophores.

Colourless forms, undoubtedly belonging to this species, have also been recorded from sea-water samples as well as their frequent occurrence being recorded from mixed cultures grown from sea-water samples.

Phagotrophy occurs very frequently (Figs. 8, 12; Fig. 18, Pl. I; Fig. 35, Pl. VI), the cell ingesting graphite, bacteria and plant cells usually up to 4 × 3 μ but in a mixed culture set up from a sea-water sample a cell of *C. strobilus* was seen to have engulfed a 15 μ long Naviculoid diatom, but the ends of the diatom were sticking out of the body. Direct examination of sea-water samples has also shown that *C. strobilus* does exhibit phagotrophy under natural conditions.

Contents of the body

Unlike *C. chiton* (1958), there is no external pyrenoid attached to the inner face of the chromatophore in *C. strobilus* and the lamellae in the chromatophores are less obvious, but, as in *C. chiton*, saturated lipid globules are present between the lamellae of the outer face of the chromatophore. Additional globules of unsaturated lipid material are also present in the body, as in *C. chiton* (1958, p. 221), but in *C. strobilus* they are generally more numerous. In the cells from an actively growing culture of *C. strobilus* there are from 1 to 4 of these lipid globules (*ca.* 0·5 μ) lying near to or against the inner face of each chromatophore (Fig. 8; Fig. 28, Pl. III; Fig. 34, Pl. IV). As growth in a culture slows down or when a culture has been grown in strong light (112 ft.c.) for 10 days the number and size of these globules increases very greatly so that a cell can contain from 10 to 20 (Fig. 11), some measuring up to 1·5 μ in diameter. This great increase in lipid material in the cell may be due to low nitrogen content of the medium (see Fogg, 1956). A control culture grown in weak light (8 ft.c.) for the same period showed that the cells still had only 1 to 4 small globules to each chromatophore.

All lipid globules stain orange to orange red with Sudan IV but only those free in the body react with osmium tetroxide becoming yellowish brown in the centre and dark brown or black on the outside. Using an unpublished method for the staining of phospholipids given to one of us (M.P.) by Dr G. Y. Kennedy of Sheffield University the following results were obtained. The small globules between the lamellae of the outer face of the chromatophores gave a reaction for lipids of the 'cephalin' group whereas the larger globules free in the body (originating from the inner storage face of the chromatophore?) sometimes gave a staining characteristic for lecithins in the centre while a layer round the outside of the globule gave a reaction for cephalins. Two or three fairly small vesicles of leucosin are generally present in this species, lying in the body usually towards the non-flagellar end of the saddle. When a cell is moving rapidly, however, the leucosin can frequently be seen pushed to the centre of the dorsal surface and forming a slight bulge in the front of the cell while the chromatophores are drawn back into the wings (Fig. 10). The cells which showed a great increase in the number and size of the lipid globules after being grown in very strong light also showed the production of very large leucosin vesicles which sometimes filled nearly half the volume of the cell (Fig. 11).

The nucleus, ovoid and up to $3 \times 2\ \mu$ in size, lies towards the ventral surface of the saddle sometimes centrally in the body but more frequently excentrically, to the left of the centre when looking down on the ventral surface.

A dark bar-shaped body lying to one side of the nucleus and visible in the living cell stains up bright red with cresyl blue and as blue-green stripes with Janus green; this is almost certainly the golgi area (Figs. 8, 9, see p. 182 and Pls. V–VII). Four to six mitochondria can be distinguished by the use of Janus green; two or four, (0·5–0·75 μ), lie fairly close to the nucleus and two others, usually larger (1–1·5 μ), lie towards the non-flagellar end of the saddle (Figs. 8, 9).

The muciferous bodies are small (*ca.* 0·25 μ) and not very conspicuous in this species (Fig. 8; Pl. V). They may be of a different structure to those described for other species (1955, 1956, 1958) since it is difficult to make them eject their contents even with the use of vital stains. *In situ* they stain a true clear blue round the outside with cresyl blue, but the contents do not appear to take up the stain. Most usually when ejection does occur with the use of dilute cresyl blue, the ejected mass appears to consist of two parts—a thicker part close to the body which becomes yellow to lime green and a much thinner mass which gradually balloons out from the thicker part and becomes a pale mauve colour; this suggests that the whole body may be ejected on a neck and that the thicker part is the organ itself—possibly not a natural procedure as the ejection is so difficult to obtain. Very occasionally, however, the ejection of short threads has been observed and, as in *C. ericina* (1956, p. 395), a small disk remains attached to the distal end of the thread.

When the cells are fixed with osmium tetroxide the contents shrink leaving the 'pellicle' or scale covering clearly visible; under high power it appears to be covered by punctae. With cresyl blue and methylene blue the pellicle frequently bursts and the cell contents are extruded leaving the 'pellicle' practically complete (cf. *C. chiton*, 1958, and see p. 181).

Reproduction

Fission in the motile phase occurs as in *C. alifera* (1956, p. 413) and takes place most frequently in late afternoon (Figs. 4, 5, 12). Non-motile stages similar to those already described for other species (1955, 1956, 1958) are then produced. The large amoeboid and walled cells with very finely lobed chromatophores, as in *C. alifera* (1956), measure from 14 × 11 to 17 × 12 μ. The ovate to sphaeroidal daughter-cells have thin walls and finely lobed chromatophores and measure from 4 × 3 to 7 × 5 μ in size. The partial but not the complete release of the motile stage from the walled daughter-cells has been observed.

OBSERVATIONS WITH THE ELECTRON MICROSCOPE—MORPHOLOGY

In addition to the observations already described from the light microscope, which include among others the measurement of relative length of the haptonema and its capacity, when alive, to become attached to a surface at any point along its length and not merely at the tip as in our previous species, the use of shadowcast whole mounts has added several significant facts by which this species is peculiar. One of these is the extreme elegance with which the haptonema uncoils, as this is expressed in dried specimens in a partly uncoiled condition. Several examples are included in Pl. I with greater detail of one of these in Fig. 27, Pl. II. The original coil itself is faintly visible in the undried cell of Fig. 18, Pl. I. The very regular configuration of two loops alternating to right and to left, often associated with the presence of apparent constrictions occurring at regular intervals along the haptonema, at first suggested to us that the whole organ might perhaps be ribbon-shaped rather than cylindrical and owe some of its properties to this cause. This suggestion is not borne out by sections, however, and therefore although in the dried condition the organ is undoubtedly flat, this flattening must in itself be attributed to the drying process. Nevertheless, it is possible that a tendency to become flattened may be greater in this than in any of our previous species, a circumstance which, if it were true, might explain the unusual ability of the haptonema to become attached throughout its length. For further discussion see p. 179.

Another peculiarity is the firmness with which the scales remain in position. In all our other species the scales have been so loosely attached both to each other and to the cell that they commonly litter the field either singly or in clusters which bear no relation to their original position. In this species scales are only rarely encountered singly. With fresh material killed directly onto the formvar film (our usual method) the scales generally appear as compact uniform sheets often still in relation to the cell body (Figs. 22, 25, Pl. II), and with the component scales held in a very regular close-packed arrangement (Fig. 25) suggesting that they are partially embedded in some kind of a matrix, the whole forming a coherent pellicle-like mat which tends to behave as a unit. When cells are not killed in this way but treated in any other of our occasional procedures such as with iodine as a preliminary to stripping from glass, the scale-mat may cohere completely to the body surface and be undetectable (Fig. 23). In embedded material, on the other hand, pieces of 'scale-mat' with the scales still in position are abundant and easily recognized (for further details see p. 181); we may therefore be certain that the close-packing seen in whole mounts is here not accidental.

There are undoubtedly two types of scale present. This is not obvious from the surface view of a scale mat which (Fig. 25) generally shows only a compact and even array of small featureless scales with raised rims. But occasionally loose scales of slightly larger diameter and with faint surface striations are encountered (Figs. 25 (top right), 26). Once seen these can with care always be detected near sufficiently dismembered cells, but they are so very thin that it is never easy to determine their finer details. Nothing can be ascertained with certainty from whole mounts about their arrangement on the cell body.

OBSERVATIONS WITH THE ELECTRON MICROSCOPE—ANATOMY

The haptonema

The great length of this organ in our present species compared with *C. chiton* (1958) is at once revealed by the clusters of much more numerous component sections which can be encountered; compare, for example, Fig. 30, Pl. IV, with Pl. IV of our previous paper. Since the structure of this organ was the most important first object of inquiry and the only one for which the American microscope was used (cf. p. 170) it will be convenient to describe it first.

As may be seen at a glance in Fig. 30, Pl. IV, the haptonema here, as in our previous species, is only about half the width of a flagellum. The actual dimensions can be directly measured either in Fig. 30 or in several of those included in Pl. VIII, all of which indicate that, disregarding obvious distortions, the average width of cross-section is of the order of 0·2 μ. It should, however, perhaps be pointed out that higher magnifications than were achieved previously have been used for most of Pl. VIII and only the inset pictures on Fig. 39 are exactly comparable with some of those reproduced on Pl. IV of our previous paper.

Within the haptonema the salient anatomical features are comparable, though not identical, with those of *C. chiton*. As before, the most conspicuous components are the three concentric membranes (using the term membrane rather loosely and without implications as to whether each is in fact single or compound) surrounding a ring of fibres (or tubes) and a central space. As before, the number of component fibres in the ring is extremely constant, having in this case been ascertained without any variation in thirteen specimens. It is, however, six and not seven as in the other species. Representative sections illustrating this number from three different cells are included in Pl. VIII (Figs. 39–41, Fig. 42 and Fig. 43 respectively), though the clearest individual section is doubtless that of Fig. 41. The diameter of each of the central fibres is of the order of 200–250 Å.

The three membranes of the haptonema wall are not all alike; the outermost one is undoubtedly compound. Its total thickness is rather less than 100 A, but when accurately cut a three-layered sub-structure consisting of two dense surface layers separated by a lighter central layer can be clearly resolved (see especially between the arrows in Figs. 41 and 40), the thickness of each of these components being of the order of 30 Å. or somewhat less. The other two membranes contained in the haptonema wall cannot be similarly resolved. They appear to be thinner than the outer triple membrane (see especially Fig. 41) and they are certainly more fragile since they often break under the action of the fixative (cf. Fig. 42). In so doing they frequently roll up and cease to be individually recognizable (see Fig. 39*c* and various parts of Fig. 40), an artifact which prevents effective discussion of the possible presence of an additional component which is not membranous. Nevertheless, certain sections (e.g. Fig. 43 uppermost LS) do suggest rather strongly that there may be a fibrous component in the haptonema wall arranged roughly at right angles to the longitudinal direction marked by the position of the central fibres. Further evidence will, however, be required before this component, if genuine, can be located with certainty.

A minor peculiarity which might perhaps be connected with the special properties referred to in the first paragraph of this section is that the central fibres seem less firmly attached to the innermost membrane than was the case in *C. chiton*. In our former species the union was so close that we were at first in doubt whether the fibres themselves were in fact thickenings or intuckings of the inner membrane and it was only in specimens considerably distended by 'blistering' that their separation could be clearly demonstrated (as in Fig. 42 here). In our present species it is the exception rather than the rule for the inner fibres to be found still touching the inner membrane, e.g. in insets *a* and *b* in Fig. 39. More usually, e.g. Figs. 41 and 43, the fibres are free from the membrane and are tending to become clustered together in the centre of the organ thus partially obliterating the cavity. Since it is an unavoidable condition of working with killed cells that we can only observe structure in relaxed coiled haptonemata and not in extended ones, it is impossible to know whether this difference from *C. chiton* is trivial or of functional significance.

The uppermost section included in the right-hand top corner of Fig. 39 perhaps represents the region of the thickened haptonema tip. It contains no interpretable structure except a solid centre surrounded by one membrane. If its nature had been more fully demonstrated, these observations would be important, but until they can be confirmed by additional, or by better, specimens we can only quote this one as possibly of this nature. We have no observations on the structure of the basal end of the haptonema.

Explanation of Plates I–IV

Chrysochromulina strobilus sp.nov.

I

Fig. 14. A cell dried on glass and photographed with the light microscope. × 1000.
Fig. 15. As Fig. 14, another cell. × 1000.
Fig. 16. Flagella and haptonema from the cell of Fig. 15 photographed by phase contrast. × 2000.
Fig. 17. The specimen of Figs. 15 and 16 after transfer to formvar, viewed with the low power of the electron microscope. Micrograph M 166.9, × 3000.
Fig. 18. A cell killed after ingesting graphite and photographed in a liquid mount under oil immersion; the central pellet of graphite and two lateral plastids appear dark, the coiled haptonema attached to the lower part of the body appears light. × 2000.
Fig. 19. As Fig. 14, another cell. × 1000.
Fig. 20. As Fig. 14, another cell. × 1000.
Fig. 21. The cell of Fig. 20 after transfer to the electron microscope. Micrograph M. 152.23, × 3000. For further details from this specimen see Fig. 25, Pl. II.

II

Fig. 22. A flattened cell seen with the electron microscope showing the layer of scales still in position outside the more opaque body. Micrograph M. 327.3, × 3000.
Fig. 23. Part of a cell with flattened appendages, stripped from a glass preparation. The partial decomposition of the haptonema has revealed a slender axis within translucent material (cf. with Fig. 27). × 10,000.
Fig. 24. Tip of a flagellum. M. 284.16, × 10,000.
Fig. 25. The edge of a scale-mat from a specimen comparable to that of Fig. 22, the cup-shaped scales still in position and apparently embedded in some amorphous material; two separate plate-scales appearing at the top of the figure. Micrograph M. 135.4, × 20,000.
Fig. 26. Free scales of the two types, the plate-scales in the upper part of the figure very transparent and difficult to record, the cup-shaped scales in the lower part of the figure projecting slightly from the surface of the mount. Micrograph M. 336.13, × 30,000.
Fig. 27. Tip of the haptonema of Fig. 21, more highly magnified, showing the swollen tip and configuration characteristic of uncoiling, in a less damaged specimen than that of Fig. 23. Micrograph M. 152.25, × 10,000.

III

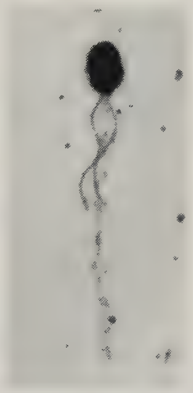
Fig. 28. Section of a cell with scales on the surface, showing nucleus (*N*) 2 plastids (*P*), a flagellar base (*fb*), two black lipoid bodies and part of a third, mitochondria, golgi area and miscellaneous vesicles. Micrograph H. 1837, × 15,000.
Fig. 29. Surface of a similar cell with scales in position (for further details see Fig. 44, Pl. VIII). Micrograph H. 1733, × 30,000.

IV

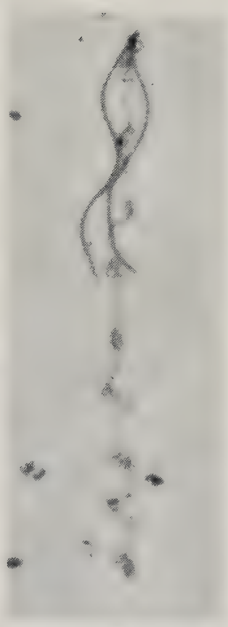
Fig. 30. Section of a cell with related haptonema (*H*) and flagella (*f*) near two detached pieces of scale mat (*S*), one inverted with respect to the other. Micrograph H. 1830, × 15,000.
Fig. 31. Part of Fig. 30 to show details of the storage region in the plastid; arrows point to sections of paired tubes which traverse it. Micrograph H. 1831, × 30,000.

14

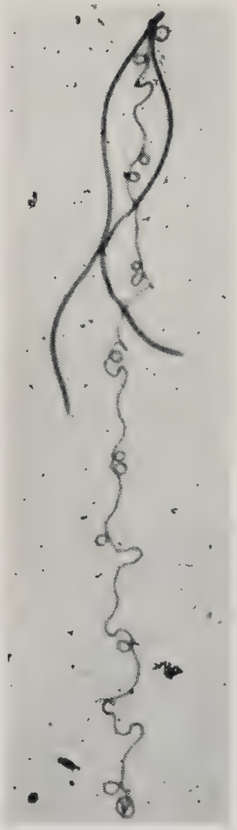
15



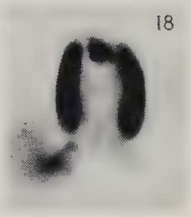
16



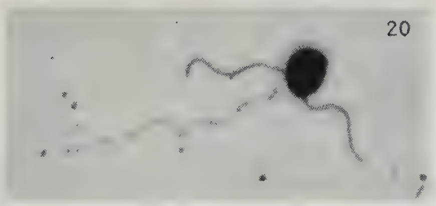
17



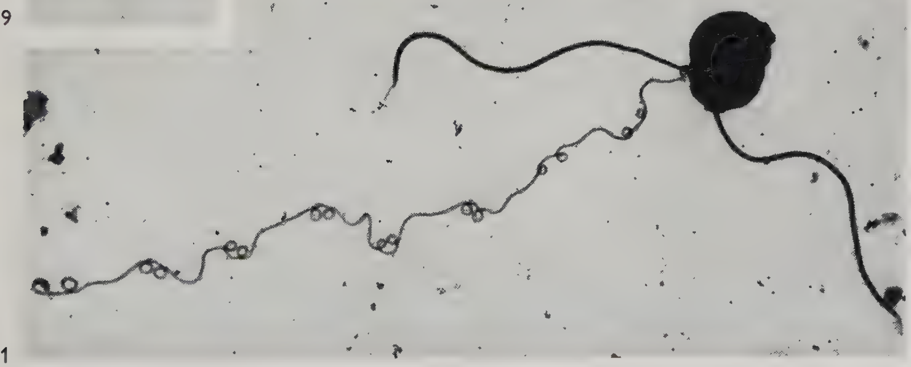
18



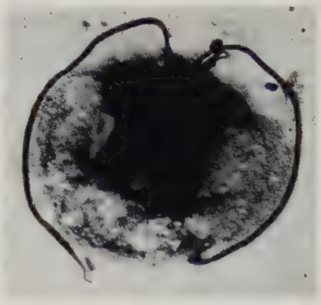
20



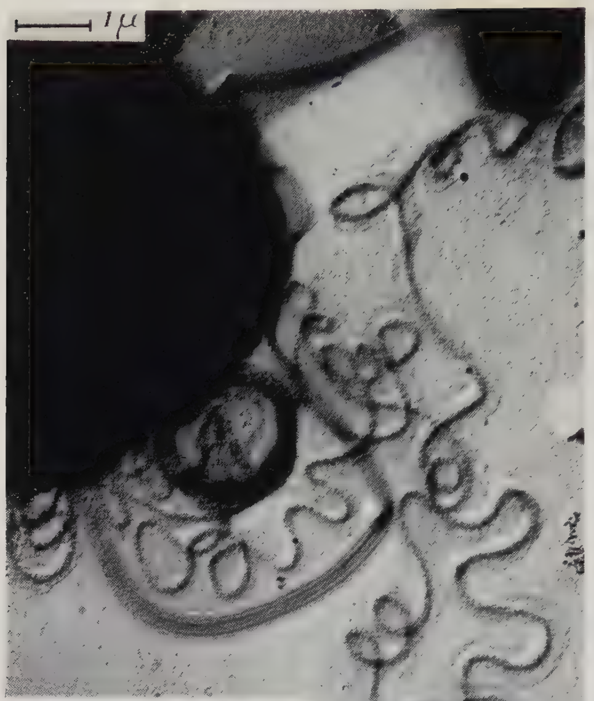
19



21

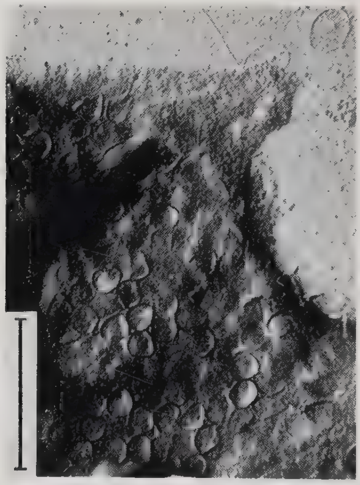


22

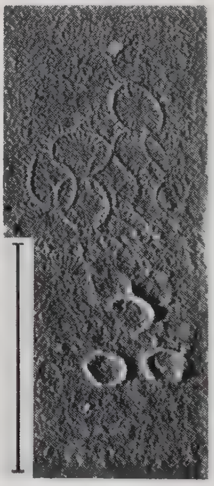


23

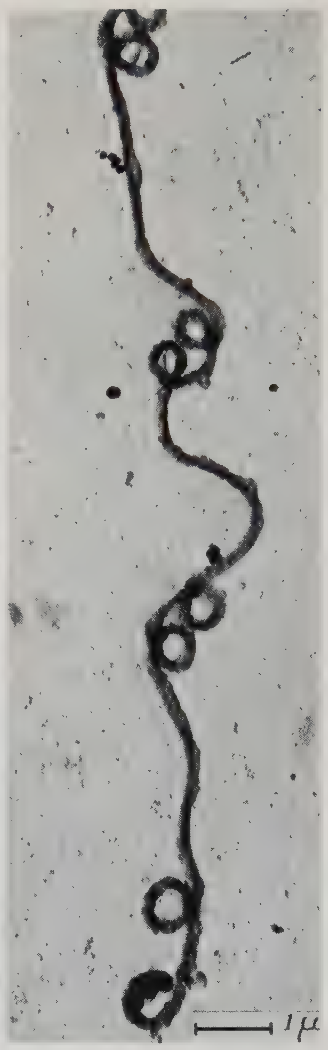
24



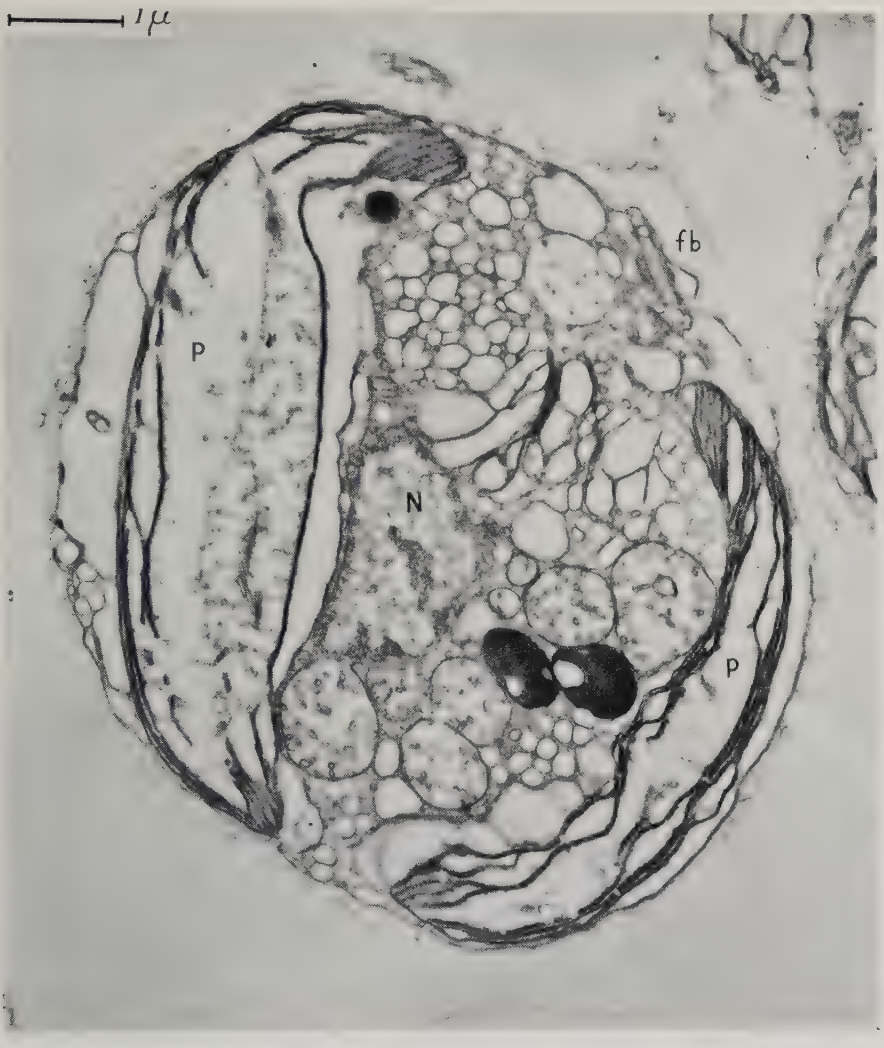
25



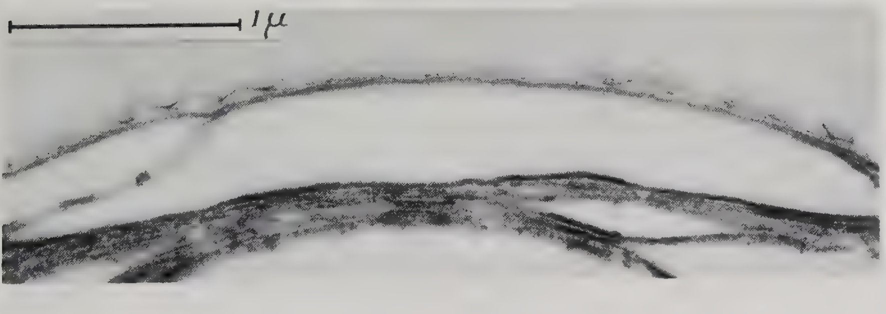
26



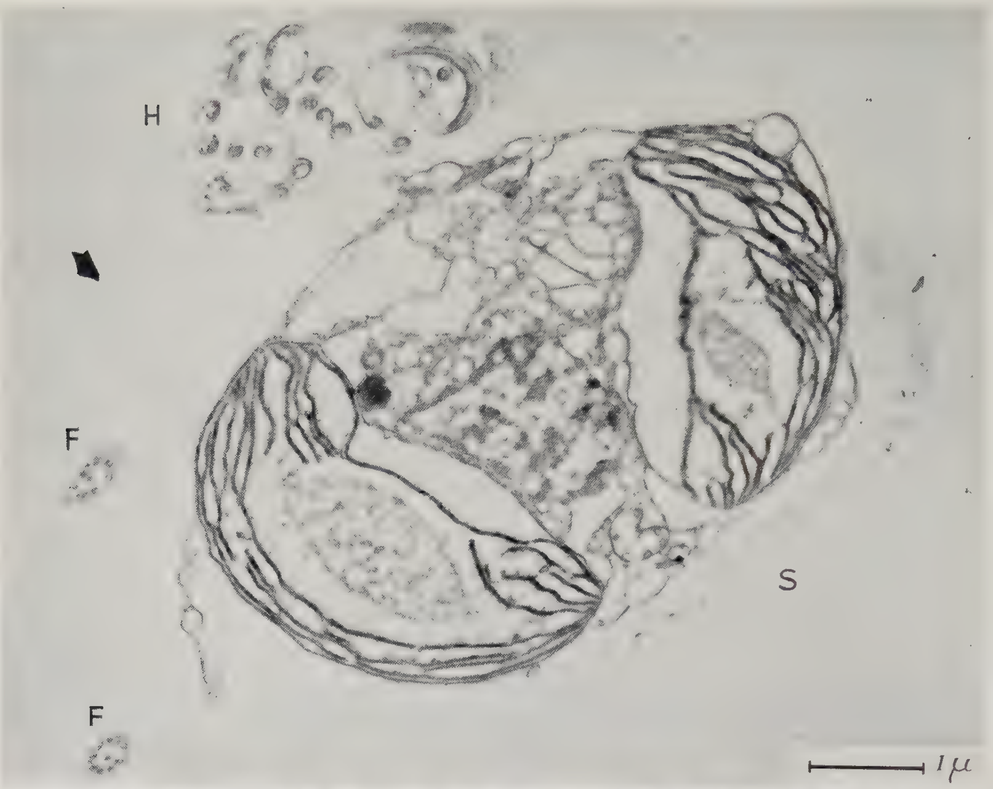
27



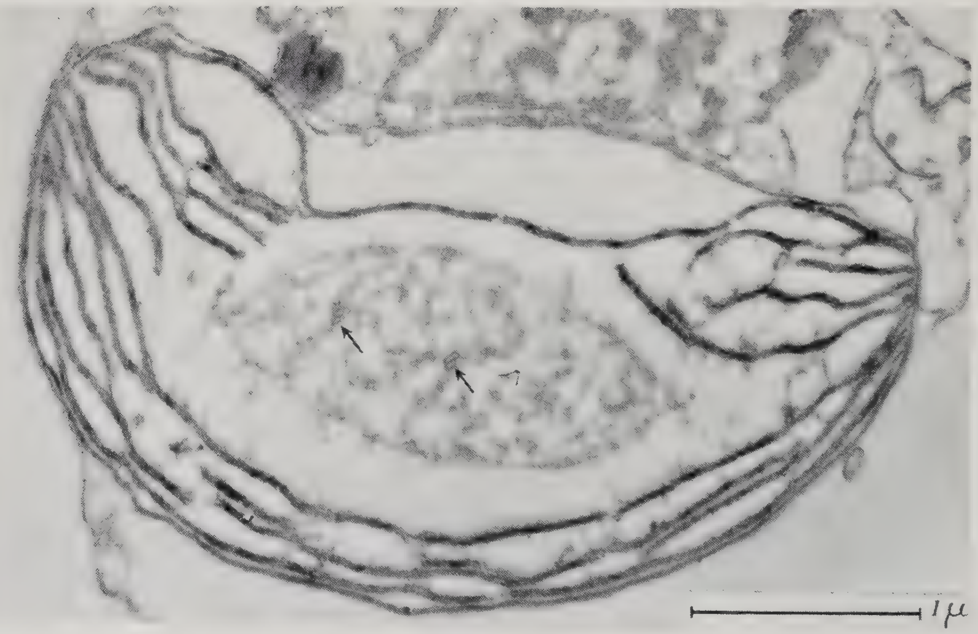
28



29



30



31

The scales

Fig. 44, Pl. VIII (for explanatory diagram see Fig. 13), shows a section of scales in position on the body at a magnification comparable to that of the adjacent haptonema sections (i.e. 60,000 as in Figs. 42 and 43). The cup-shaped scales with conical thickened base are arranged in a very regular layer, as was to be expected from the external view. Beneath them are the plate scales, also in a single layer; each is a very delicate sheet with a slight central thickening and a rim projecting up from both surfaces like the edge of a coin. Other less highly magnified sections showing the scales still in position on the cell are contained in Pl. III and elsewhere.

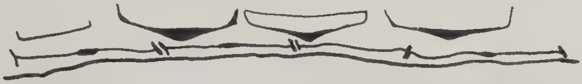


Fig. 13. Diagram traced over a photographic print to show the arrangement of scales in Fig. 44, Pl. VIII, magnification of the diagram × 80,000.

Detached scales, still arranged in sheets, are common. Two such sheets, one inverted with respect to the other, are situated near the letter S on Fig. 30, Pl. IV. Neither can be directly related to the adjacent cell since the sheet nearest to the body surface is upside down with respect to it. When cut transversely the conical cup-scales, arranged side by side, produce an effect as of a delicate zig-zag line at this low power. The amorphous material partially investing the scales on dried material is no longer visible in sections but this is likely to be due to solution in the methacrylate since the regular array of scales in each sheet is undisturbed. This can be seen no less clearly when a sheet is cut tangentially as in the top corner (right) of Fig. 32, Pl. V. In such an aspect the scales appear circular with a central opaque area; there is again no sign of intercalary material between them but they are in exactly the same serial arrangement as in Fig. 25, Pl. II.

Beneath the scales is a membrane, as in *C. chiton*, and sometimes though not always a layer or layers of small vesicles. The diameter of these vesicles is of the same order as that of the scales. Since there were no superficial vesicles in *C. chiton* as small as this we may perhaps find this fact to be significant when developmental processes can be more seriously considered.

The internal organs

A glance at the low-power views of complete sections reproduced in Pls. III–V will sufficiently indicate the general positions of the more important body organs. The chromatophores, nucleus, mitochondria and food vacuole are labelled as such in Fig. 32, Pl. V. In addition the lipid bodies in the cell interior and the muciferous bodies on the surface are conspicuous by their opacity, a property connected with their chemical effect on the osmic fixative. Leucosin vacuoles or equivalent spaces bounded by a membrane appear empty.

Some of these organs or organelles resemble those of *C. chiton* sufficiently closely to be passed over without further comment; the nucleus, mitochondria and food vacuole fall into this category. Differences of a fairly striking kind do nevertheless exist and since we are still without sufficient knowledge to be able to distinguish characters which may be of generic importance from others which only delimit taxa of a lower order, it will be necessary to enumerate differences as fully as possible. The following comments will therefore be restricted to this topic.

The pyrenoid

The most conspicuous difference between the internal organs of *C. chiton* and our present species concerns the pyrenoid. In *C. chiton* this was a sub-spherical body filled with dark contents and attached by a narrow neck to the inner face of a chromatophore. No such bodies can be detected in our present species. Instead there is an internal storage region within the chromatophore, often of considerable extent and containing similar dark contents. A good example is illustrated in Pl. IV and parts of others will be detectable elsewhere. The stored material is traversed by some highly characteristic narrow paired tubes which are almost certainly continuous with some of the lamellations of the pigmented part of the chromatophore although we have not on this occasion reproduced any micrographs illustrating this continuity. Examples of the tubes in question, cut in slightly oblique TS are indicated by arrows in Fig. 31, Pl. IV.

The flagellar bases

These have not been studied in great detail here but it is necessary to locate them before attention can be given to the organelle to be discussed in the next paragraph. They differ a little from the situation in *C. chiton* by the extreme obliquity with which they are inserted. This is clearly indicated in Fig. 28, Pl. III, where a flagellar base (fb) in almost median LS is lying almost parallel to the cell surface. This is a highly characteristic position and we have never encountered in this species the almost vertical orientation of flagella to the surface which was illustrated, for example, in Fig. 34, Pl. IX, of our previous paper. When both flagellar bases are contained in the same section they make a very obtuse angle with each other as in Fig. 34, Pl. VI.

Golgi

Immediately below the attachment of the flagellar bases is an area occupied by vesicles of the characteristic kind previously diagnosed as golgi. The golgi area is commonly bounded by the nucleus, mitochondria and chromatophores on the sides towards the cell (e.g. Fig. 28, Pl. III and Fig. 32, Pl. V), though it is never separated from the flagellar bases by any of these organs, and it is not delimited by a membrane. When cut tangentially or merely grazed, only the compacted vesicles and traces of flattened paired membranes are visible (e.g. Figs. 28, 32). When cut more nearly centrally, however, a surprising complexity of structure is revealed. Pls. VI and VII are inserted to illustrate this.

In Pl. VI are two cells cut in planes approximately at right angles to each other but both passing through the central region of the golgi area. A more highly magnified view of one of these cells is reproduced in Fig. 38, Pl. VII, while the two upper figures on Pl. VII are similarly magnified views of two different sections from one specimen resembling Fig. 34 in plane of cutting. Pls. VI and VII thus illustrate the golgi structure in three different cells.

The central part of the golgi area is most easily interpreted as a cavity occupying the concave side of the strongly curved outer paired membranes (flattened cisternae) into which many of the inner paired membranes are budding off vesicles. These vesicles are commonly somewhat distorted and perhaps burst as a fixation artifact leaving numerous fragments of membrane distributed through colourless liquid. For further discussion see p. 185.

Muciferous bodies

The only additional details to add about these organelles are firstly that they are not very numerous. A section displaying as many as appear in Fig. 32, Pl. V, is an exception selected for reproduction for this reason; the more usual condition is as in the other plates in which these bodies are only encountered here and there or not at all. In structure they recall those of *C. chiton* though they may perhaps be simpler. There is no suggestion here of any obvious modification of the outer wall, an undischarged body merely projecting a little above the surface of the cell (Fig. 33) without other detectable structural features. The contents appear slightly darker than in our micrographs of *C. chiton*, but a minor difference in fixation procedure could perhaps explain this.

DISCUSSION

While the more obvious comparisons, namely those with *C. chiton* (Parke, Manton & Clarke, 1958), have already been made, a few general comments remain. It is clear that the structural differences recorded fall into two rather different categories. On the one hand there are clearly determined divergences as in the pyrenoids and numerical details within the haptonema, on the other are observations which could depend in part on fuller knowledge of our present species. It may be recalled that for our previous paper there was only very limited access to high resolution microscopes and the amount of material surveyed was in consequence severely restricted. We cannot therefore be quite certain without further investigation of *C. chiton* whether, for example, the very peculiar structure encountered in the centre of the golgi area in our present species is actually absent from our previous one or whether we failed to detect it for the reason given.

Explanation of Plates V–VIII

Chrysochromulina strobilus sp.nov.

V

Fig. 32. Section of a cell to show muciferous bodies (black) in the outermost layer. In addition the nucleus (*N*), mitochondria (*m*), a food vacuole (*FV*), spaces which could have contained leucosin, golgi vesicles near the base of the appendage (uncertain whether this is a flagellum or a haptonema). Arrow in the top right-hand corner points to a scale mat seen in surface view; elsewhere scales in position on the body and sections of bacteria in the ground. Micrograph H. 1637, × 18,000.

Fig. 33. Part of the surface of a similar cell to show the way in which full muciferous bodies (dark) project above the cell surface. Micrograph H. 1969, × 20,000.

VI

Fig. 34. Section of a cell to show golgi structures between the two plastids (*P*), and below the flagellar bases (*fb*). In addition (dark) lipid bodies, mitochondria and vesicles. Micrograph H. 1706, × 15,000.

Fig. 35. A similar cell cut in a plane roughly at right angles to the preceding showing the nucleus (*N*), a large food vacuole (*FV*) and the golgi area immediately above it; for further details see Fig. 38, Pl. VII. Micrograph H. 1833, × 15,000.

VII

Figs. 36, 37. Two sections, some distance apart, of a cell cut somewhat as in Fig. 34, to show the structure of the golgi area when cut near its edges (Fig. 36) and more centrally (Fig. 37). Signs of the flagellar bases (*fb*) visible in Fig. 36. Two fat bodies and the edges of adjacent plastids, arranged as in Fig. 34 visible laterally (see especially Fig. 36), in addition several mitochondria (*m*) and scattered vesicles. Micrographs H. 1619 and H. 1624, × 25,000.

Fig. 38. Detail of the golgi area in Fig. 35 more highly magnified, lettering as in that figure. Micrograph H. 1834, × 25,000.

VIII

Fig. 39. General view of a haptonema with inset details of three component sections (a, b, c) from the places marked by the upper arrows; lower arrow a significant region for Figs. 40 and 41; *fb* a flagellar base on what is probably the subtending cell. Micrographs RS. 80, × 25,000, and RS. 378, × 50,000 (insets).

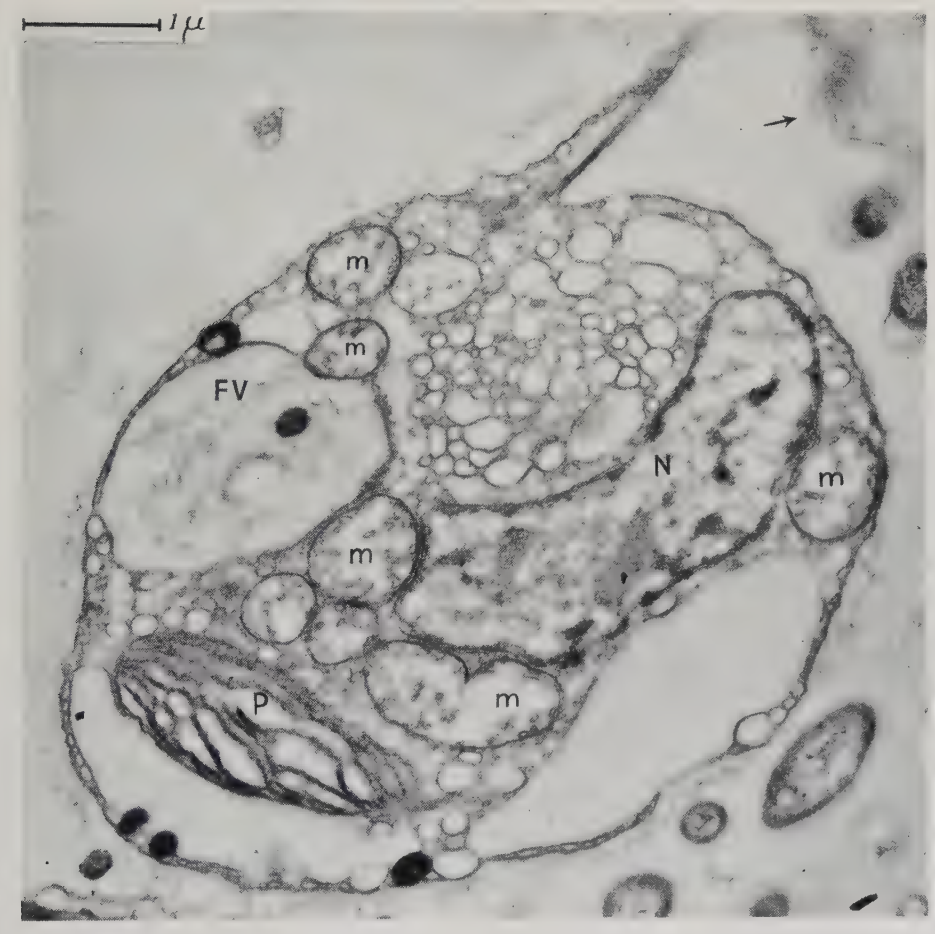
Fig. 40. Part of another section through the haptonema of Fig. 39 more highly magnified. Micrograph RS. 390, × 70,000.

Fig. 41. Part of the preceding more highly magnified and placed in a different attitude on the page; the six central fibres and three concentric membranes very clearly displayed with also (between the arrows) evidence that the outermost membrane is double. Micrograph RS. 390, × 100,000.

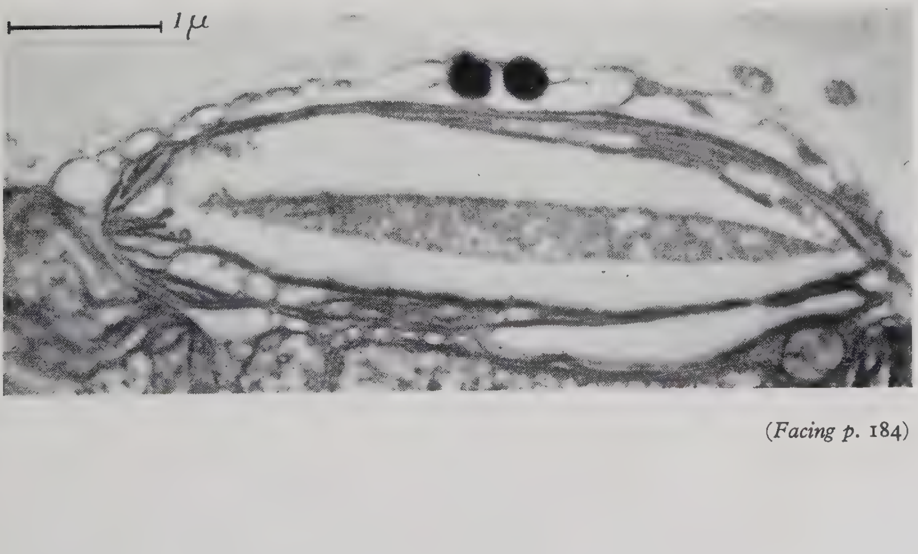
Fig. 42. Part of another specimen exhibiting 'blistering' but showing the three membranes and six central fibres very clearly when the inner membranes have split. Micrograph RS. 400, × 60,000.

Fig. 43. Part of another specimen to show a similar general anatomy in TS (lower arrow) and parts of an LS (upper arrow). Micrograph H. 1611, × 60,000.

Fig. 44. Section showing the two types of scale in position on a cell, the cup-shaped scales forming an outer layer and the plate-shaped scales below. Micrograph RS. 60, × *ca.* 60,000.

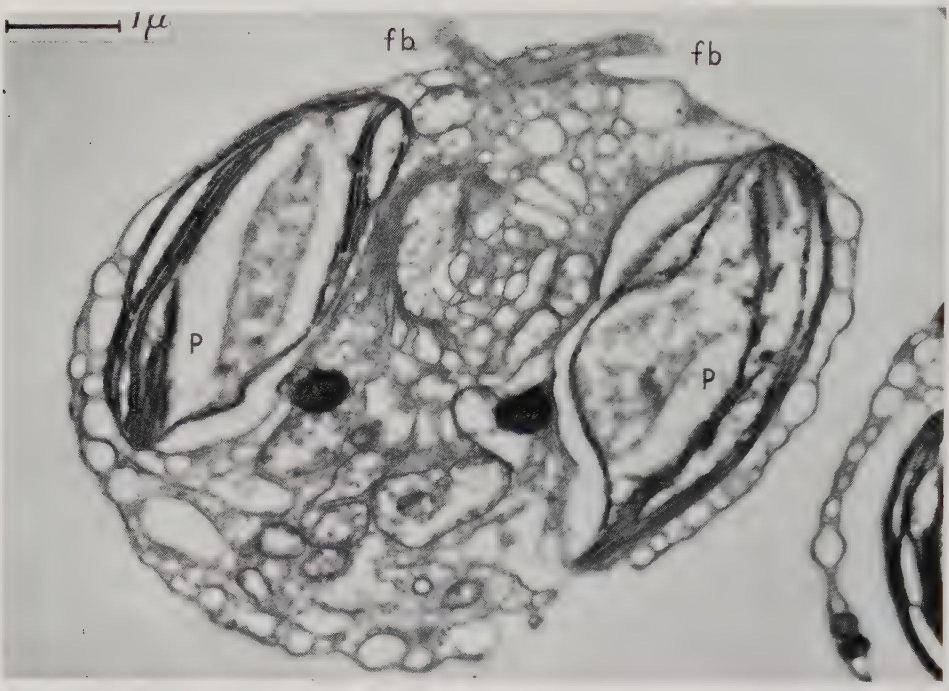

1 μ
m
m
FV
N
m
m
P
m

32

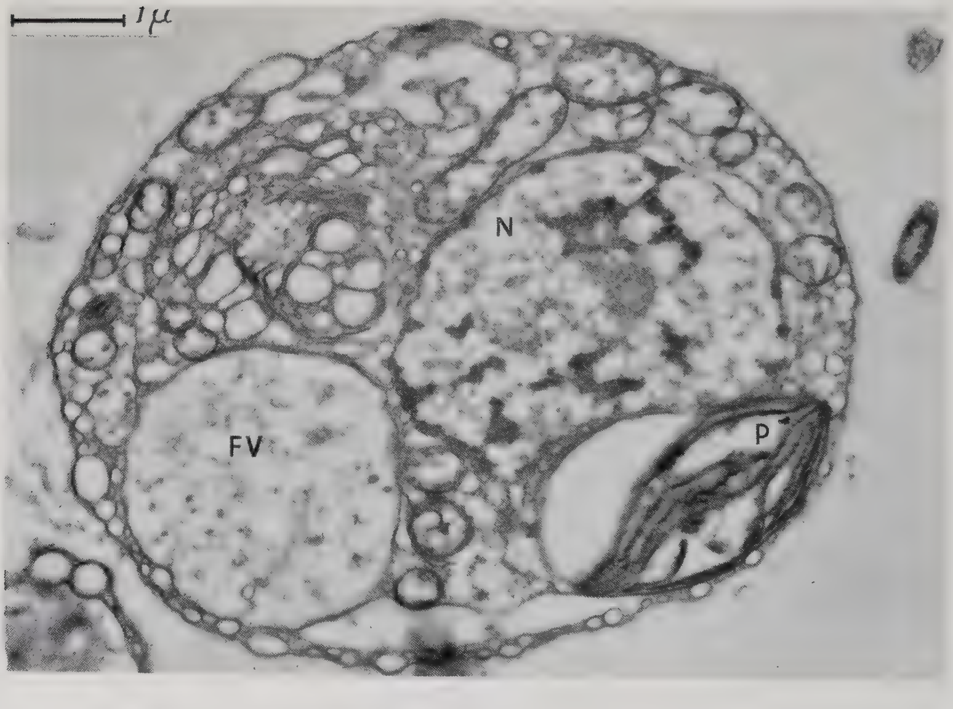


33

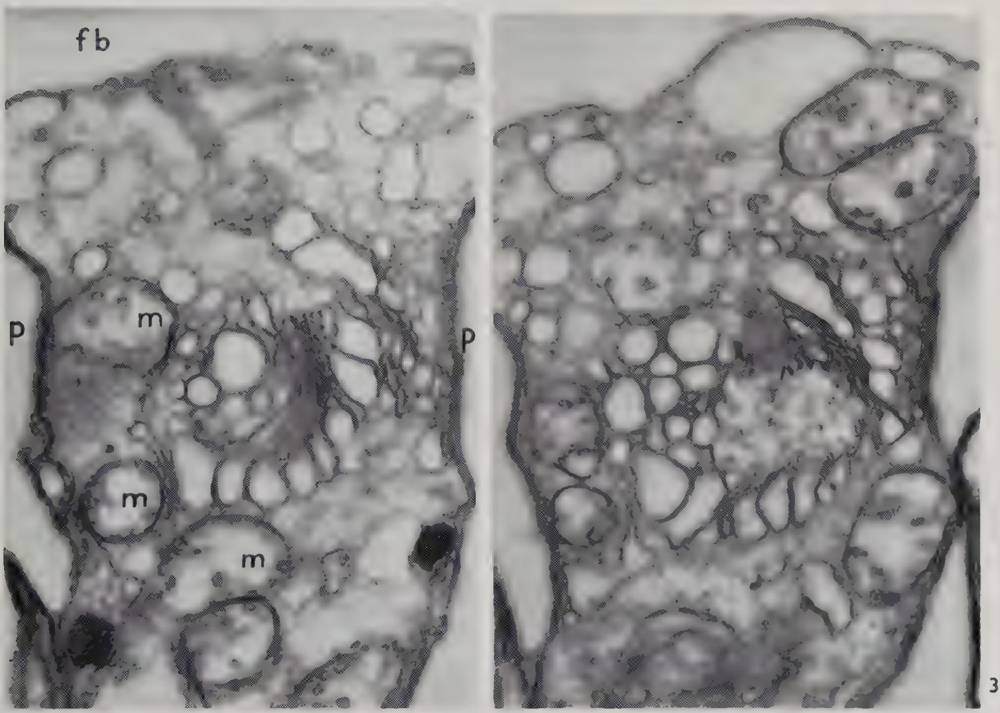
(*Facing p.* 184)



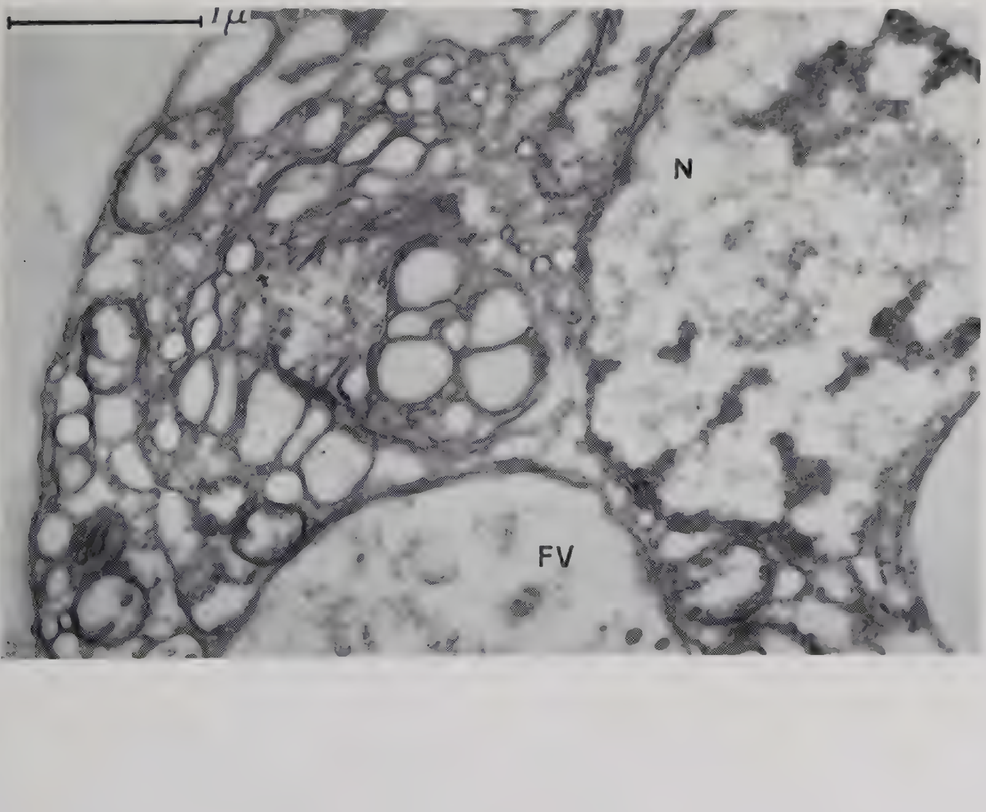
34



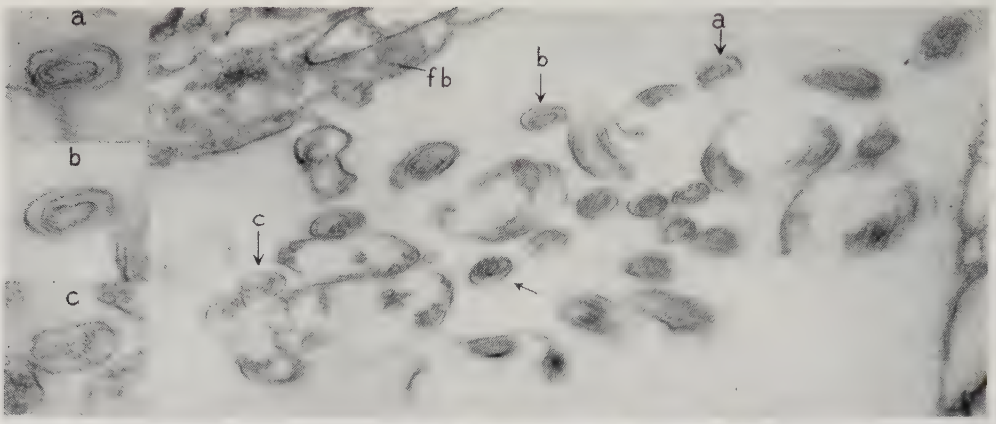
35



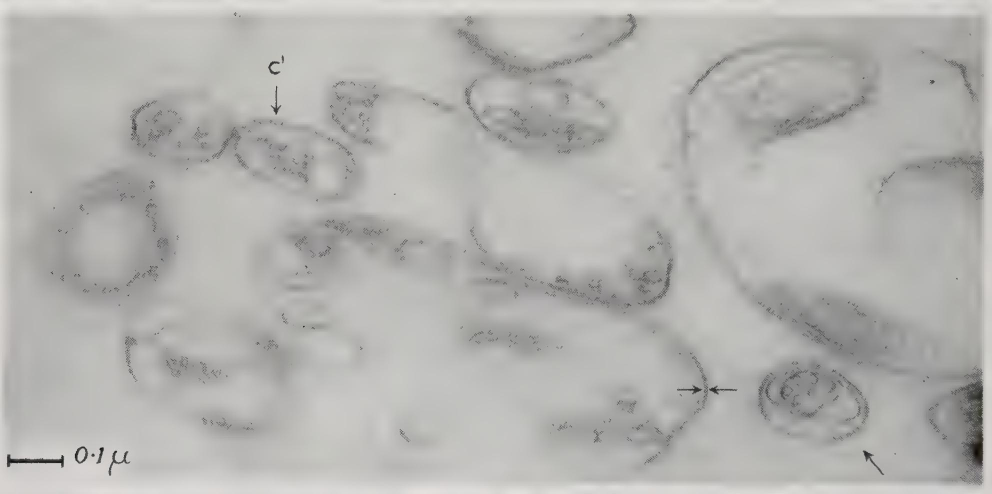
37



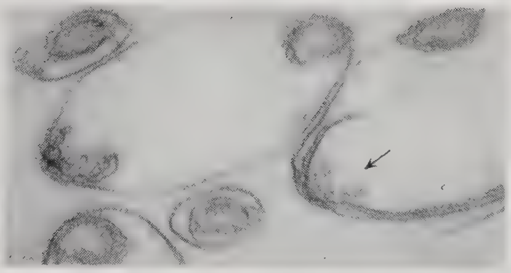
38



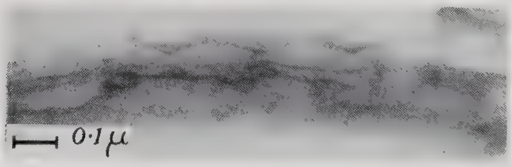
39



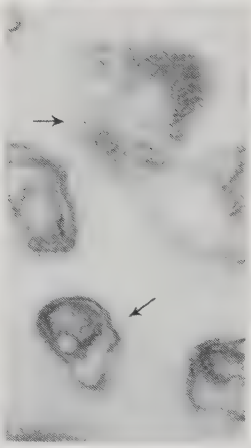
40



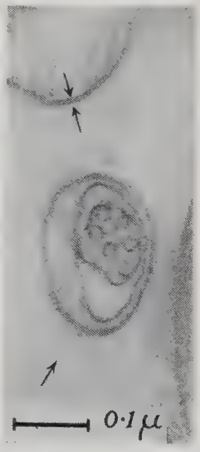
42



44



43



41

There is, however, no doubt that the observations recorded for the centre of the golgi area in our present species are unusual, and for this reason we have at once taken steps to ascertain whether or not we are here dealing with an idiosyncrasy confined to one species. Preliminary sections of two other of our previously described species, *C. ericina* and *C. ephippium* of Parke, Manton & Clarke, 1956, have, however, at once revealed exactly comparable details, and since at least one of these species seems to be a more favourable object for study than our present one we propose to defer further discussion of this organ until more evidence can be surveyed.

We are on firmer ground with regard to the pyrenoid and haptonema for which positive differences from *C. chiton* have been demonstrated. A pyrenoid of the type encountered here recalls very closely that recently described for *Hydrurus* by Hovasse & Joyon (1957). We can say further (Manton, unpublished) that similar pyrenoids, differing only in minor features of size and position, are present also in *C. ericina* and *C. ephippium.* It is therefore possible that in this character *C. strobilus* rather than *C. chiton* may represent the norm within the group.

With regard to the haptonema structure, external comparisons are not yet possible since knowledge is at present limited to the two species under discussion. Comparison between these species is, however, instructive in more than one way. The numerical difference in fibre number, 7 in *C. chiton* and 6 in *C. strobilus*, is not to be explained away as a sampling error since both numbers have been determined with equal certainty through a range of specimens. Each number seems to have the constancy of a specific character, but this only underlines the fundamental difference between the haptonema and a flagellum for which complete uniformity of internal structure prevails, not merely from species to species but right through the plant and animal kingdoms. The resemblances, on the other hand, are no less important since here we may believe that the two species are both contributing essential information towards a descriptive understanding of this complex and peculiar organ, which though still incomplete, is significantly enhanced by the new facts contributed by *C. strobilus.* The triple structure of the outermost membrane of the haptonema wall is most unlikely to be a specific peculiarity confined to *C. strobilus* since similar membranes are now known to be a normal covering to many external protoplasmic organs, e.g. cilia and flagella. This very fact, however, indicates fairly clearly that the special properties possessed by the haptonema are likely to be determined by the internal parts about which less is known.

With regard to the scales the presence of two distinct types arranged one beneath the other in separate layers seems to remove entirely the possibility of continuous production of scales from the body surface at all times. It seems necessary to conclude that scale production must in some way be a cyclic phenomenon, a conclusion which was also, though less definitely, formulated in connexion with the rather different scale arrangement in *C. chiton.*

SUMMARY

A new species of *Chrysochromulina*, *C. strobilus*, characterized by a very long haptonema capable of attaching at any point along its length and by very small scales of characteristic pattern has been described. The anatomy of the haptonema consists, as in a previous species, *C. chiton*, of three concentric membranes surrounding a ring of fibres and a central space. The outermost membrane is three-layered and there are traces of some intercalary material between this and the middle membrane. The number of fibres in the inner ring is 6 and not 7 as in *C. chiton*; each fibre is of the order of 200–250 A. in width. The organs internal to the cell show two major differences from those of our previous species, notably in the pyrenoid and golgi area. The pyrenoid is inside the chromatophore; its structure is described. The golgi area shows a central structure which is so unusual that a full description is reserved until more species have been examined.

REFERENCES

Fogg, G. E., 1956. Photosynthesis and formation of fats in a diatom. *Ann. Bot.*, Vol. 20, pp. 265–85.

Hovasse, R. & Joyon, L., 1957. Sur l'ultrastructure de la Chrysomonadine *Hydrurus foetidus* Kirchner. *C.R. Acad. Sci., Paris*, T. 245, pp. 110–13.

Parke, M., Manton, I. & Clarke, B., 1955. Studies on marine flagellates. II. Three new species of *Chrysochromulina*. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 579–609.

—— —— —— 1956. Studies on marine flagellates. III. Three further species of *Chrysochromulina*. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 387–414.

—— —— —— 1958. Studies on marine flagellates. IV. Morphology and micro-anatomy of a new species of *Chrysochromulina*. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 209–28.

APPENDIX

Table 1. RECORDED DISTRIBUTION OF *CHRYSOCHROMULINA STROBILUS* SP.NOV.

Date	Position		Depth
	N.	W.	(m.)
12. xi. 48	50° 19·5′	04° 10′*	10
1. ii. 49	50° 19·5	04° 10′	10
8. vi. 49	50° 19·5′	04° 10′	10
12. iv. 50	49° 25′	03° 47′†	0·5
13. iv. 50	50° 15′	04° 13′	0·5
4. v. 50	50° 15′	04° 13′	0·5
9. v. 50	49° 21′	04° 54′†‡	0·5
23. viii. 50	Tamar Estuary off Torpoint		—
20. ix. 52	Off the Azores§		0·5
17. i. 56	50° 02′	04° 22′	20
29. v. 56	50° 06′	04° 21′	5
29. xi. 56	50° 07′	04° 23·5′	0·5
2. i. 57	50° 03′	04° 04·5′	0·5
19. vi. 57	50° 09′	04° 15′	—
16. vii. 57	50° 02′	04° 22′	0·5
24. vii. 57	50° 11′	04° 14′	0·5
17. ix. 57	50° 02′	04° 22′	0·5, 5, 10, 20, 70
17. ix. 57	50° 06′	04° 21′	0·5
	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
	50° 20′	04° 10′	0·5
15. x. 57	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
6. xi. 57	Tamar Estuary off Forder Mill		—
3. xii. 57	50° 02′	04° 22′	5, 10, 20, 70
3. xii. 57	50° 06′	04° 21′	0·5
3. xii. 57	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
	50° 20′	04° 10′	0·5
21. i. 58	50° 02′	04° 22′	20, 50
21. i. 58	50° 06′	04° 21′	0·5
	50° 15′	04° 13′	0·5
	50° 15′	04° 11′	0·5
	50° 20′	04° 10′	0·5
18. ii. 58	50° 02′	04° 22′	0·5 5, 10, 20, 50, 70
18. ii. 58	50° 06′	04° 21′	0·5
	50° 15′	04° 13′	0·5
18. ii. 58	50° 18′	04° 11′	0·5
	50° 20′	04° 10′	0·5
16. iii. 58	47° 40′	07° 13′	10, 20
18. iii. 58	47° 46′	07° 05′	20
19. iii. 58	50° 02′	04° 22′	0·5, 5, 10, 20, 50, 70
19. iv. 58	46° 30′	08° 00′	10
20. iv. 58	47° 30′	07° 18′	10
	47° 38′	07° 10′	10, 225
21. iv. 58	47° 46′	07° 05′	10, 20, 163
22. iv. 58	50° 02′	04° 22′	0·5, 5, 10, 20, 50, 70
20. v. 58	50° 02′	04° 22′	5, 10, 20, 50, 70
20. v. 58	50° 06′	04° 21′	0·5
	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
	50° 20′	04° 10′	0·5
10. vi. 58	50° 02′	04° 22′	0·5, 5, 10, 15, 20, 50, 70
10. vi. 58	50° 06′	04° 21′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
9. vii. 58	50° 02′	04° 22′	0·5, 5, 10, 70
9. vii. 58	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
26. viii. 58	50° 02′	04° 22′	0·5, 5, 20, 30, 50, 70
26. viii. 58	50° 06′	04° 21′	0·5
1. x. 58	50° 02′	04° 22′	0·5, 5, 10, 20, 50
22. x. 58	50° 02′	04′ 22′	0·5, 10, 20, 50, 70
	50° 20′	04′ 10′	0·5
18. xi. 58	50° 02′	04° 22′	0·5
	50° 06′	04° 21′	0·5
	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5

* Strain of *Chrysochromulina strobilus* (Plymouth no. 4) isolated from this sample.

† Samples brought in by 'Sir Lancelot'.

‡ Type culture strain of *Chrysochromulina strobilus* (Plymouth no. 43) isolated from this sample.

§ Sample brought in by 'Discovery II'.

TABLE 2. SEASONAL DISTRIBUTION OF *CHRYSOCHROMULINA STROBILUS* SP.NOV.

	International Station E 1								Plymouth Laboratory Stations				
									L6	L5	L4	L3	L2
Depth (m) ...	0·5	5	10	15	20	30	50	70	0·5	0·5	0·5	0·5	0·5
Date													
17. ix. 57	3	3	3	—	3	—	0	1	2	3	2	2	3
15. x. 57	0	0	0	—	0	—	0	0	0	1	1	0	0
6. xi. 57	1	—	0	—	0	—	—	—	—	—	—	—	—
3. xii. 57	0	2	3	—	5	—	0	2	1	1	1	1	1
21. i. 58	0	0	0	—	2	—	1	0	1	0	2	1	2
18. ii. 58	2	3	3	—	4	—	1	3	2	0	3	2	3
20. iii. 58	3	3	4	—	4	—	5	5	—	—	—	—	—
22. iv. 58	6	6	6	—	4	—	3	3	—	—	—	—	—
20. v. 58	0	2	3	—	3	—	2	2	3	4	4	2	1
10. vi. 58	5	5	6	4	3	—	2	4	5	0	1	1	0
9. vii. 58	1	2	1	0	0	—	0	1	0	2	2	2	0
26. viii. 58	4	1	0	—	1	3	3	3	4	0	0	0	0
1. x. 58	2	2	3	—	1	—	1	0	—	—	—	—	—
22. x. 58	2	0	1	—	1	—	1	1	0	0	0	0	2
18. xi. 58	1	—	0	—	0	—	0	0	2	1	1	4	0

6, min. no. *C. strobilus* per l.	1000
5, min. no. *C. strobilus* per l.	800
4, min. no. *C. strobilus* per l.	600
3, min. no. *C. strobilus* per l.	400
2, min. no. *C. strobilus* per l.	200
1, min. no. *C. strobilus* per l.	10
0, Absent from sample	

E 1	50° 02′ N., 04° 22′ W.
L 6	50° 06′ N., 04° 21′ W.
L 5	50° 11′ N., 04° 13′ W.
L 4	50° 15′ N., 04° 13′ W.
L 3	50° 18′ N., 04° 11′ W.
L 2	50° 20′ N., 04° 10′ W.

J. mar. biol. Ass. U.K. (1959) 38, 189–220
Printed in Great Britain

ON THE BIOLOGY OF *PANDALUS BOREALIS* KRØYER, WITH REFERENCE TO A POPULATION OFF THE NORTHUMBERLAND COAST

By J. A. Allen
Dove Marine Laboratory, Cullercoats

(Text-figs. 1–14)

The deep sea prawn, *Pandalus borealis* Krøyer, is fished extensively in Norwegian, Swedish and Greenland waters, some 4000 metric tons being caught annually, having a value of about £800,000. Its biology has been investigated by various workers in these countries. Comprehensive accounts have been given by Wollebaek (1903), Hjort & Ruud (1938), Rasmussen (1953) and Horsted & Smidt (1956). Despite this, relatively few populations have been sampled at regular intervals throughout a year. In high latitudes where much work has been carried out, ice, for varying lengths of time, prevents stocks from being sampled. Rasmussen (1942, 1949, 1953) has shown that the life history of *P. borealis* varies with locality in a range from southern Norway to Spitsbergen. When *P. borealis* was found in numbers in deep water off the Northumberland coast advantage was taken to investigate the biology of the species at the southern limit of its eastern Atlantic distribution and to compare the results with those of other workers. Knowledge of the biology of the prawn now covers the whole of its north-south distribution and some account of this is given. In addition, the study gives information on a population that has not been fished commercially and provides a more complete picture of sex reversal than has been obtained hitherto.

I am particularly indebted to Dr A. Ritchie of the Scottish Home Department Laboratory, Aberdeen, who has allowed me to use his data on the distribution, size and sex of *P. borealis* in British waters. I am also indebted to Dr H. O. Bull for many suggestions, for records of distribution and for temperature and salinity data. I wish to thank Mr R. Harrison, skipper of the R.V. 'Alexander Meek' for collecting in all weathers, and my wife for her criticism.

DISTRIBUTION

P. borealis occurs in both Pacific and Atlantic oceans (Fig. 1). Ekman (1953) refers to it as having a discontinuous north Atlanto-Pacific distribution and as a discontinuous circum-*boreal* species. Earlier records of its distribution are given by Rathbun (1904) and Hofsten (1916), the latter giving detailed

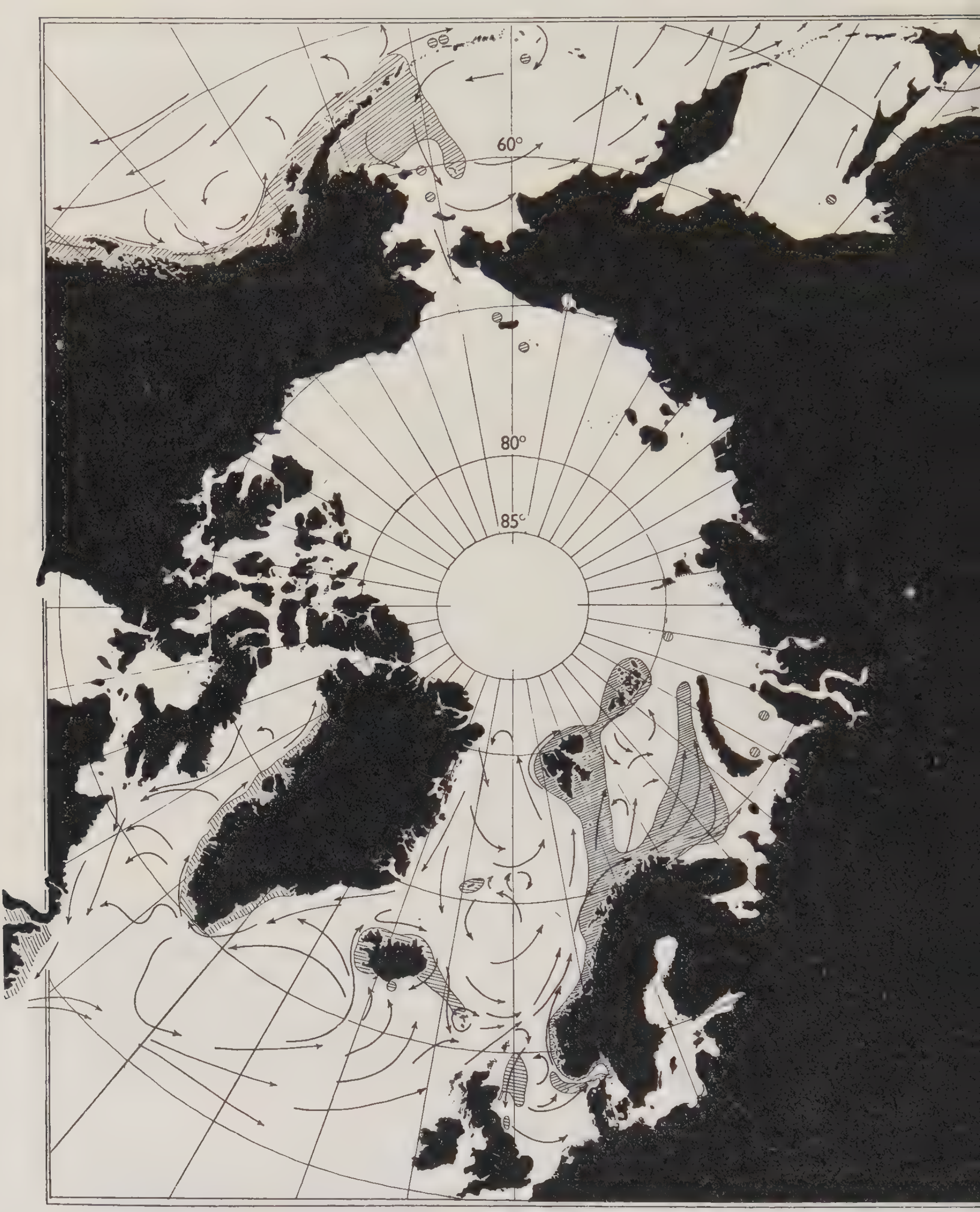


Fig. 1. Distribution of *Pandalus borealis* (shaded areas). Arrowsi ndicate the general direction of flow of the surface currents. Records of Berkeley (1930), Blacker (1957), Bjørk (1935), Gorbunow (1934), Grieg (1925, 1926*a*, *b*, 1932), Heegaard (1941), Hjort & Ruud (1938), Hofsten (1916), Horsted & Smidt (1956), Hynes (1929), Johnson & Lindner (1934), Poulsen (1946), Pruter & Harry (1952), Rasmussen (1942, 1953), Rathbun (1904, 1929), Sivertsen (1932), Stephenson (1935) and the present work.

maps and references. The present maps (Figs. 1, 2) bring knowledge of its distribution up to date, the authorities being listed in the legends to the figures.

Knowledge of the distribution of the prawn in the Pacific and off the eastern seaboard of America has been little extended since the work of Rathbun (1904). Rathbun (1929), Hynes (1929), Berkeley (1930), Johnson & Lindner (1934) and Hjort & Ruud (1938) all refer to its distribution but give few new localities. *P. borealis* is common off the coasts of Alaska and to the south as far as the Columbia River (lat. 46° N.). It is also common in the eastern part of the Bering Sea and off the Aleutian Islands. To the north of the Bering Straits it has been recorded in latitude 73° off Vrangelye Island. It is much less common off the western coasts of the Pacific but is reported from Japanese waters at least as far south as latitude 45° N. In the eastern Atlantic *P. borealis* occurs in large numbers in the Gulf of Maine and off Cape Cod. The most southerly record is in latitude 41° N. There is a curious lack of records between Cape Cod and the southern tip of Greenland (Fig. 1), although it has been presumed to occur (Johnson & Lindner, 1934; Hjort & Ruud, 1938). On the west coast of Greenland there are dense populations supporting a lucrative fishery (Horsted & Smidt, 1956), but there are no records of its occurrence on the west side of the Davis Strait (see p. 194). Miss V. Brawn of St Andrews Biological Station, N.B., Canada, in a personal communication, states that it is present in the Gulf of St Lawrence but has no record from farther north.

Large populations of *P. borealis* occur in eastern Atlantic waters off the Norwegian coast and as far north as Franz Josef Land (lat. 82° N.), but no farther east than longitude 79° E. They occur off the east coast of Greenland as far north as latitude 66° N., and off the coasts of Spitsbergen, Jan Mayen, Iceland and the Faroes. To the south of Norway the prawn is present in the Skagerak and Kattegat, but no farther south than latitude 56° N. It occurs in deep water (>80 m) in the North Sea (Fig. 2). A single specimen has been recorded from south of the Dogger Bank in latitude 54° 24′ N. (Wedmeyer, 1912), but it is doubtful whether there is any permanent population south of latitude 55° N. *P. borealis* was first recorded off the Northumberland coast north-east of Coquet Island by Todd in 1907 (Norman & Brady, 1909). A specimen taken off the Scottish coast in 1904 by the 'Michael Sars' (Grieg, 1926*b*, see Appendix I) appears to be the first British record. Neither Bell (1853) nor Calman (1899) list *P. borealis*. Although Jorgensen (1923) records larvae as present in plankton samples from the Northumberland area this is probably incorrect (see p. 208). As yet there is no published record of larvae being taken from the North Sea by continuous plankton recorder surveys (Rees, 1952, 1955). Through the kindness of Dr I. Gordon, Dr A. Ritchie and Dr H. O. Bull unpublished records from British waters from 1930 to the present date are available (see Appendix I).

Information on *Pandalus borealis* is sufficiently complete to give a reliable picture of its ecology over the whole of its eastern Atlantic distribution. Temperature, salinity, substratum, and, possibly, depth are limiting factors in its distribution. Adult prawns have been taken from water with temperatures ranging from – 1·68° to 11·13° C (Fig. 3). Horsted & Smidt (1956) have

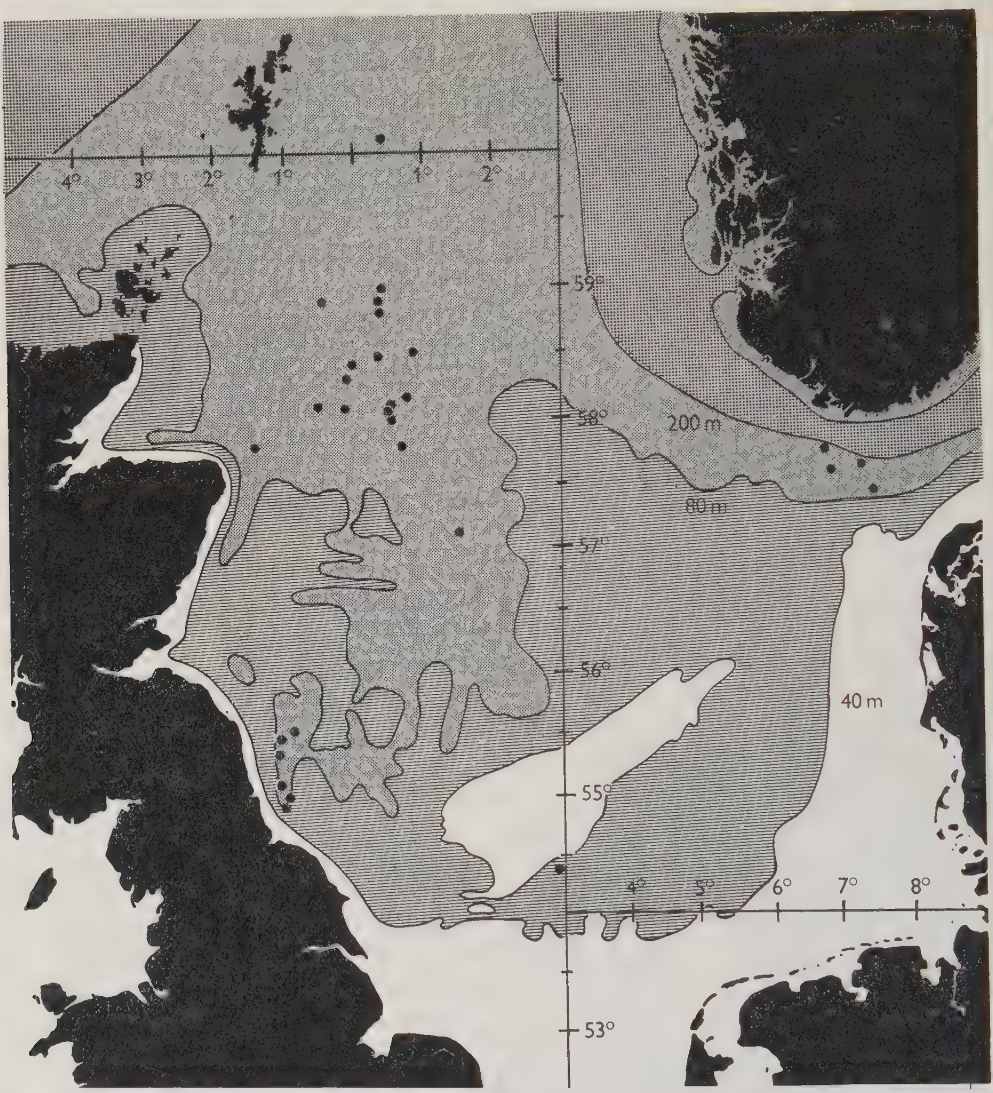


Fig. 2. The distribution of *Pandalus borealis* (black circles) in the North Sea relative to depth. Records of Allen (unpubl.), British Museum (unpubl.), Bull (unpubl.), Grieg (1926*b*), Poulsen (1946), Ritchie (unpubl.), Todd (Norman & Brady 1909) and Wedemeyer (1912).

shown that prolonged (2 months) temperatures of less than −1° C are deleterious to prawns and caused mass mortality in the stocks at Holsteinborg during 1948–49 with the consequent failure of the fishery for the following 5 years. Ekman (1953) refers to *P. borealis* as a boreal species (as its name suggests) and presumes that it owes its existence in high arctic waters to transport from warmer regions, notably by means of the Gulf Stream and the North Atlantic Drift. He questions whether the species can propagate in high latitudes. While it undoubtedly owes its existence in high arctic waters to the north-east of Spitsbergen and the north of Novya Zemlya to the warm North Atlantic current, Rasmussen (1942) has shown that the Spitsbergen population reproduces. Similarly, populations off the West Greenland coast (lat. 75° N.) at temperatures close to limiting also reproduce (Horsted & Smidt, 1956). It is a true arctic-boreal species with little or no 'sterile expatriation area'. There is little doubt that the extremely cold water of the Labrador current is one factor that prevents the establishment of stocks on the west side of the Davis Strait. Temperature is probably the most important factor limiting distribution in high latitudes. It has been assumed that the upper limiting temperature is 8° C (Rasmussen, 1953, and others), although Poulsen (1946) showed that adults can live at 9·5° C and schizopod stages at 14° C in Danish waters. The present study shows that the adults can live and breed in temperatures as high as 11·1° C. The range in temperature of the water of the prawn grounds off the Northumberland coast during the present investigation was from 6° to 11·1° C and during the period July to December was 9° C or above. There are no records of temperatures at the southern limit of distribution off the Pacific and Atlantic coasts of America, but general charts, e.g. Schott (1942), indicate temperatures of approximately 8° C. It

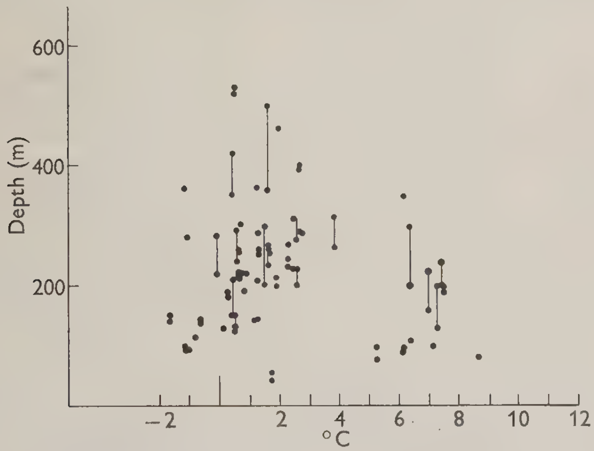


Fig. 3. Records of the depths of various populations of *Pandalus borealis* plotted against temperature of their environment. Authorities as listed in the legend to Fig. 1.

appears likely that *P. borealis* off the Northumberland coast is living at the extreme upper limit of its temperature range. Apart from Poulsen (1946) who records larvae from water at 14° C in the Kattegat, there are no records of a high temperature sterile expatriation area.

Adult *P. borealis* are restricted to water of fairly high salinity. Although Poulsen (1946) found larval stages in salinities of 32‰, only in Oslo Fjord (33–33·6‰; Hjort & Ruud, 1938) has the prawn been recorded in salinities of less than 34‰. All other records range from 34·1 to 35·7‰ and only two records exceed 35‰. The salinity of the deep water off the Northumberland coast varies from 34·47 to 34·87‰. The species is stenohaline, therefore salinity, in addition to temperature, must limit its spread along the Labrador coast and in high latitudes in the eastern Pacific.

P. borealis is restricted to soft mud in deep water where it feeds on small bottom animals and detritus. There is some evidence (Wollebaek, 1903; Hjort & Ruud, 1938) that diurnal vertical migrations occur. Horsted & Smidt (1956) believe that they take a significant quantity of their food above the bottom and that night migration is related to corresponding migrations of food animals. Hjort & Ruud (1938) and Horsted & Smidt (1956) give detailed lists of the associated fauna and show that it is similar throughout the prawn's geographical range. A similar association occurs in the North Sea. Here *P. borealis* is present on soft muds below 80 m, and the following are the common associated species: *Crangon allmani* Kinahan, *Pandalus montagui* Leach, *Spirontocaris lilljeborgii* Danielssen, *Nephrops norvegicus* L., *Calocaris macandreae* Bell, *Abra nitida* (Müller), *Thyasira flexuosa* (Montagu), *Glycera rouxi* Audouin & M. Edwards, *Amphiura chiajei* Forbes.

Distribution in depth varies from 20 to 900 m, but most records fall between 80 and 650 m. Records taken from Rasmussen (1953) and Horsted & Smidt (1956) show that the higher the latitude the deeper is the densest population. Few prawns are found in the deepest water (>600 m) of the Skagerak, although conditions appear suitable (Poulsen, 1946). The most suitable substrata may not necessarily be in deeper water in the north. Salinity and temperature will be as significant as type of substratum in determining the depth of the population. Thus values of temperature and salinity are relatively high and constant in deep water (350–600 m) in high latitudes, while in shallower water limiting values may be exceeded.

There is a relationship between the distribution of *P. borealis* and the warm current systems (Fig. 1). Horsted & Smidt (1956) show that its distribution on the west coast of Greenland is closely related to the Irminger current. There is clear correlation between the eastern Atlantic populations and the ramifications of the Gulf Stream and also between the eastern Pacific populations and the warm northerly coastal currents off the west coast of America. The prawn does not occur west of the Orkney–Shetland channel in British waters, and it is possible that the North Sea stocks originate from Faroe or

Norwegian populations (Fig. 1). There appear to be suitable grounds for *P. borealis* off the west coasts but it is possible that the larvae are restricted to the northern fringe of the Gulf Stream and will not be carried to these waters.

METHODS

The prawns used in this study were all obtained from the same position—13 nautical miles east of Blyth harbour entrance. A 9 ft beam trawl fitted with a King's Lynn type prawn trawl was used. All hauls were of 20 min duration, the same trawl being used throughout the work. The position worked is also a hydrographical station, and Dr H. O. Bull has kindly allowed his records to be used. Many of the latter were taken at the same time as the prawn haul.

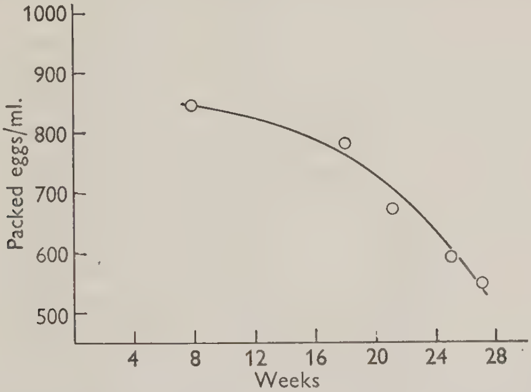


Fig. 4. Numbers of packed eggs/ml. (see text) plotted against age of the eggs.

The catch was examined alive and sorted. *P. borealis* was fixed in 4% neutral formalin in sea water and later transferred to 70% alcohol. All specimens were sexed and the carapace length from the posterior limit of the eye socket to the extreme posterior lateral edge of the carapace measured. Measurements were taken to the nearest millimetre. The correction factor for this population, that figure which when multiplied with carapace length gives total length, is 5·05 (extreme limits 4·8–5·2). This is a higher factor than that of Horsted & Smidt (1956). The latter using the same measurement of carapace obtained a factor of 4·6–4·7.

Eggs, when present, were counted, sized and examined for degree of development. The method used for counting was that of Rasmussen (1953). Eggs of a small number of specimens were counted and then hand-centrifuged in a calibrated tube and the packed egg volume noted. A conversion factor (packed eggs/ml.) is calculated and the egg numbers of the remaining specimens derived from packed egg volumes. As the egg volume increases with increasing age (Fig. 4), a new conversion factor has to be calculated for each sample.

Specimens, male, female and intersex, were selected from each size-group of each sample and the secondary sexual characters examined. The endopodite of the first pleopod, the appendix interna of the second pleopod and appendix masculina, if present, were drawn accurately with the aid of a squared eye-piece graticule. The same objective and eyepieces (magn. × 5 and × 10 respectively) were used throughout the work. Gonads from the selected specimens were removed for sectioning and the sections stained with Mallory's Triple Stain, and Eosin and Fast Green.

SEX REVERSAL AND GAMETOGENESIS

Most *Pandalus* species are protandrous hermaphrodites (Berkeley, 1930; Kubo, 1951; Pike, 1952; Pruter & Harry, 1952; Mistakidis, 1957). *P. borealis* is no exception and some account of this is given by Berkeley (1930), Leopoldseder (1934) and Jägersten (1936). External changes in the endopodite of the first pleopod are well described, but accounts of changes in form of the appendix masculina and the changes in the gonads and the relation of the latter to the changes in the external sexual characters are far from complete.

Rasmussen (1953) and Horsted & Smidt (1956), in addition to showing that life span varies in different populations, find that the ages at which maturity and sex changes occur also vary. Their data has been compared with that obtained in the present study in Table 1. In high latitudes prawns may not mature as males until their third year and change sex in the sixth year, while in the North Sea maturity is reached after 18 months and more than 30% may never show male characters. Sex reversal in those showing male characters occurs before they are 27 months old.[1] Northumberland prawns do not live for more than 38 months. Table 1 shows that intermediate populations (Eids, Ofoten and Mist Fjords) are also intermediate in respect of their age at maturity and at sex change. Thus the life history of the North Sea population most closely resembles that of the Oslo Fjord population. Hjort & Ruud (1938) found in the latter population that approximately 5% never function as males.

Hjort & Ruud (1938), discussing sex change in the southern Norway populations, suggest a 'labile equilibrium' between male and female potentialities where the age of female maturity depends on the age at which the male tendencies are repressed. Jägersten (1936) from work on *P. borealis* from Gullmar Fjord suggests that all individuals are hermaphroditic and have both male and female potentialities. Jägersten recognizes three categories: (1) primary females, in which potentiality is repressed so early that male characters never appear; (2) secondary females, in which male potentiality is repressed soon after male characters have appeared (these animals mature as females in their second year); and (3) hermaphroditic females, in which male potentialities are repressed after functioning as a male. Rasmussen (1953) shows a definite relationship between size, maturity and sex (see Table 1); normally prawns less than 70 mm total length at the breeding season are immature, while those greater than 115 mm total length are females. Few prawns above 110 mm total length function as males and only a few mature females were recorded below 90 mm. Rasmussen (1953) was able to predict the percentage of immature, males and females from the size range of the age-group at the beginning of the breeding season. The size range of the North Sea prawn at the commencement of the first breeding period (1½ years) is 76–96 mm and approximately 35% are primary or secondary females (see p. 196), i.e. a much higher proportion than the 5–10% predicted from Rasmussen's data. As Rasmussen (1953) points out, variations in relationship can be found in different localities, although it seems likely that this particular variation is significant. In the Northumberland population the slight increase in the

---

[1] The age of the prawns is calculated from the date at which all the larvae are hatched, i.e. the end of the second week in April (see p 206).

TABLE 1. PERCENTAGE COMPOSITION OF POPULATIONS OF *PANDALUS BOREALIS*

The percentages of immature, mature male and mature female *P. borealis* at the breeding seasons of various populations. Total length measurements in mm are given in parentheses. Scandinavian data extracted from Rasmussen (1953) and Horsted & Smidt (1956).

Locality	Age yr.	% immature (length)	% mature ♂ (length)	% mature ♀ (length)
1. Northumberland	1½	—	63·5 (76–90)	36·5 (81–96)
2. S. Norway		—	82–99·5 (80–94)	0·5–18 (90–101)
3. Eids and Ofoten Fjords		0–0·2 (58)	99·8–100 (58–85)	—
4. Mist Fjord		100 (50)	—	—
5. W. Greenland and Jan Mayen		100(49)	—	—
6. Spitsbergen		100 (40)	—	—
1. Northumberland	2½	—	—	100 (100–126)
2. S. Norway		—	0–78·5 (96–111)	21·5–100 (120–130)
3. Eids and Ofoten Fjords		—	16–88·5 (92)	11·5–84 (100–103)
4. Mist Fjord		—	100 (77)	—
5. W. Greenland and Jan Mayen		100 (69)	—	—
6. Spitsbergen		86–99 (64)	1–14 (78)	—
2. S. Norway	3½	—	—	100 (130–140)
3. Eids and Ofoten Fjords		—	—	100 (122–143)
4. Mist Fjord		—	100 (96)	—
5. W. Greenland and Jan Mayen		—	100 (94)	—
6. Spitsbergen		—	100 (87)	—
2. S. Norway	4½	—	—	100 (150–159)*
3. Eids and Ofoten Fjord		—	—	?*
4. Mist Fjord		—	—	100 (113·5)
5. W. Greenland and Jan Mayen		—	71 (115)	29 (126)
6. Spitsbergen		—	100 (104)	—
4. Mist Fjord	5½	—	—	100 (120)
5. W. Greenland & Jan Mayen		—	—	100 (139)
6. Spitsbergen		—	—	100 (118)
6. Spitsbergen	6½	—	—	100 (121)
6. Spitsbergen	7½	—	—	100 (140)
6. Spitsbergen	8½	—	—	100 (> 140)*

* Very few specimens.

number of females during the first 18 months of the life of the year group (Fig. 5) corresponds with sex reversal to secondary females. Although it is the largest of the immature male prawns of each sample that show reversal, there does not appear to be a minimum size below which reversal does not take place. Increased growth rate, rather than the attainment of a certain size, appears to be related to sex reversal. Observations support a theory of hormonal control (see pp. 205 and 214).

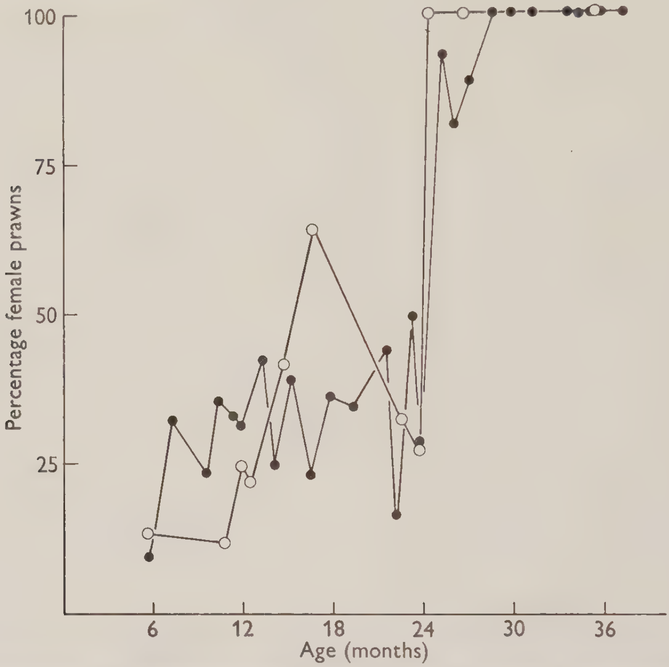


Fig. 5. The variation in the percentage of female *Pandalus borealis* during the life of a year group. Calculated from Northumberland (dots) and Scottish data (rings).

It might be expected that where late reversal of sex takes place both eggs and sperm may be produced by the same animal at 18 months. No prawn was taken that carried eggs and also possessed male external sexual characters, but sections of one prawn, 14 months old, male, with the terminal setae of the appendix masculina slightly shorter than normal, showed proliferating oocytes as well as primary spermatocytes (see p. 204). Similarly, reversal might be so retracted in the case of a male that the prawn acts as a male for a second time at 2½ years. Hjort & Ruud (1938) report four such animals from southern Norway, all very large specimens 116–126 mm. Two specimens showing unmodified external male sexual characters 4 months after egg-laying were taken, but sections of the gonads showed that oocytes only were present. It is unlikely that specimens of the Northumberland population act twice as males.

Secondary sexual characters

The change in form of the endopodite of the first pleopod and the appendix interna and appendix masculina of the second pleopod that occurs with sex reversal in the Northumberland population is shown in Fig. 6. The endopodite and appendix interna of the primary females (outlined, but not hatched) gradually increase in size throughout the life of the prawn. There is little change in shape, only the tip of the endopodite becoming more attenuated with age. The form and arrangement of the setae are not shown. 'Breeding dress' is assumed at the moult preceding egg-laying. In this condition the setae are numerous, long, feathery and adapted for the attachment of eggs. Høglund (1943) gives an excellent description of the change. In Fig. 6 those pleopods shown stippled are drawn from prawns in breeding dress.

The male endopodites (black in Fig. 6) bear the copulatory organ which projects just below the apex. A number of small hooks or cincinnuli are present on the tip of the organ, 4–6 in young prawns and more than 10 in older specimens. Below the copulatory organ are 3 or 4 small spine-setae and the remainder of the setae on the endopodite are typically long and pinnate. The above details are very similar to those described by Mistakidis (1957) for *P. montagui*. In young prawns the copulatory organ is relatively long and slender and extends beyond the apex. In older males the organ is considerably broader in relation to its length and barely extends beyond the more defined apex. The male endopodite is somewhat larger than that from a female of the same age, the change in shape with age being shown in Fig. 6. The appendix masculina, situated between the side of the endopodite of the second pleopod and the appendix interna, was present in all male prawns and no difficulty was experienced in sexing the youngest specimens. The appendix masculina of 6-month prawns is a small rounded projection bearing two or three small apical setae. The projection grows rapidly in length and by 12 months is almost as long as the appendix interna, the number of setae increasing to five terminal and three lateral. The setae are almost as long as the appendix masculina itself. Maximum size is reached at about 16 months. Atrophy occurs after the sperms have been released.

The form of the external characters of the males after spawning may change at 22 months or as late as 28 months (Fig. 6 hatched appendages). The copulatory organ becomes progressively thinner and the apex of the endopodite more pointed. There appear to be three transitional stages before the copulatory organ is finally lost. In many specimens the tip of the organ is lost at the third stage, the base remaining. The appendix masculina is slower to disappear than the copulatory organ and at least one extra moult is necessary before it too is lost, i.e. at least five moults are required for the complete reversal of the external male characters. The first indication of change in the appendix masculina is reduction in size and number of the setae, followed by the

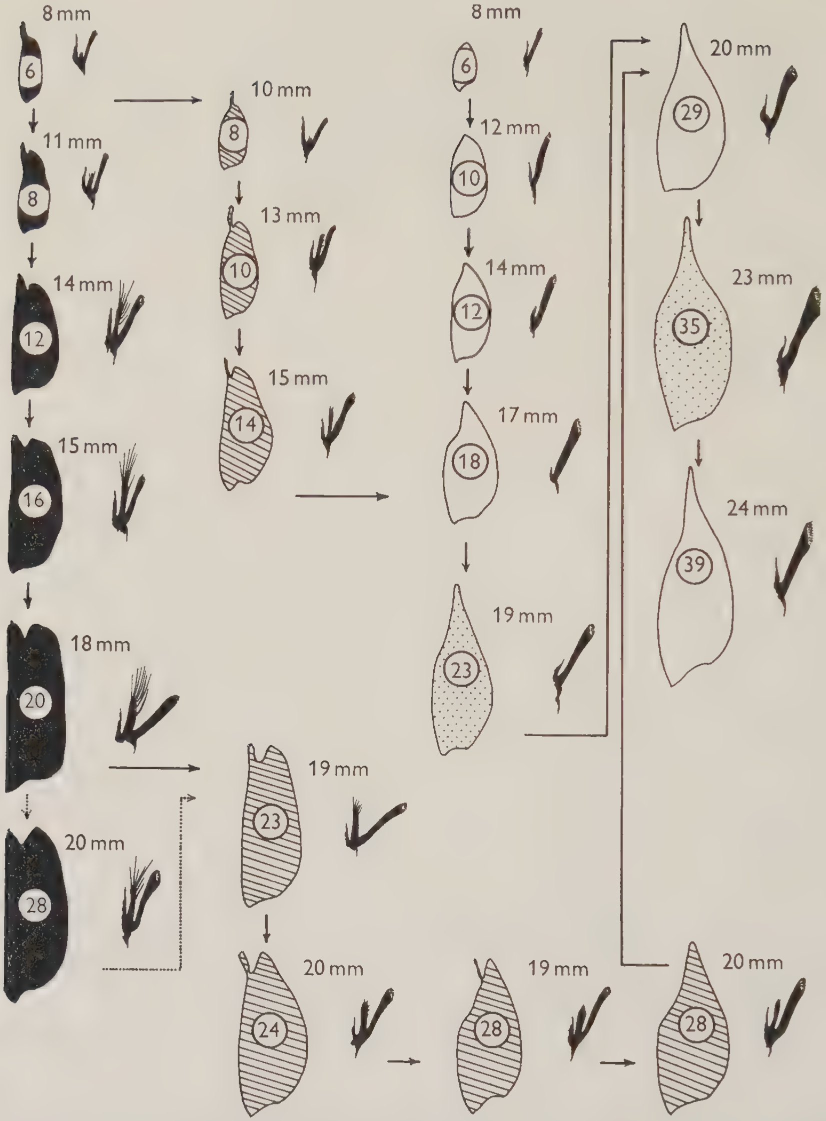

8 mm
6
11 mm
8
14 mm
12
15 mm
16
18 mm
20
20 mm
28
10 mm
8
13 mm
10
15 mm
14
19 mm
23
20 mm
24
8 mm
6
12 mm
10
14 mm
12
17 mm
18
19 mm
23
19 mm
28
20 mm
29
23 mm
35
24 mm
39
20 mm
28

Fig. 6. To show the changes in form with increasing age of the endopodite of the first pleopod and the corresponding appendix interna and appendix musculina of the second pleopod of *Pandalus borealis* from the Northumberland population. Age in months is given in the ring in each endopodite and the carapace length (mm) above each figure. Male endopodite, black; transitional, cross-hatched; female, outlined; those in breeding dress, stippled. Arrows indicate sequence.

gradual reduction in size of the appendix. Transition stages in immature prawns were found on re-examination of Northumberland samples. The youngest was 8 months old. A similar series of stages were found to those described above, although the appendix masculina never becomes fully setose (Fig. 6).

Histology of gonads

The general morphology of the gonads has been described by Wollebaek (1903), Berkeley (1930), Leopoldseder (1934) and Jägersten (1936). They are elongate, paired, tubular structures joined by a bridge about a third the distance from the anterior end. The vas deferens and oviduct join the gonad mid-laterally, the oviduct being anterior to the vas deferens (Berkeley, 1930, p. 130). Sections, mainly transverse, were cut from some 70 gonads representative of all ages and external sexual characters. The maximum oocyte diameter was recorded for each gonad to give information on oocyte growth (Fig. 7).

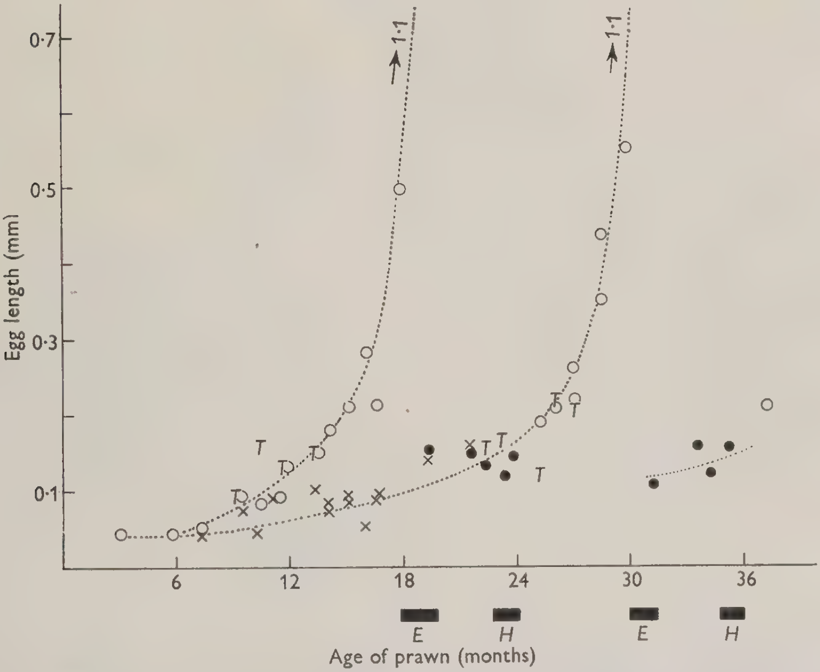


Fig. 7. Graph to show the increase in length of the oocytes (dotted line) with the age of the prawn. The plotted lines may be continued upwards linearly, and at a size of about 1·1 mm the oocytes have developed into eggs. The symbols indicate the sex of the prawns; ●, female carrying eggs on the pleopods; ○, female not carrying eggs; × male; *T*, transitional; *E*, egg-laying; *H*, hatching of larvae.

Primary females. The maximum oocyte diameter of the youngest female prawn taken (3 months) was 0·04 mm. The oocytes occupy approximately half the ovary. The remainder of the ovary is filled with small cells which are probably early follicle or interstitial cells. Aoto (1952) describes a similar condition in *Pandalus kessleri.* The proliferating strand of oocytes is not central but on the inner, ventral side of the gonad. During the next 3 months there is little increase in oocyte size but proliferation continues until much of the ovary is filled. There appear to be fewer interstitial cells, but this may possibly be due to the attenuation of their cytoplasm and new arrangement.

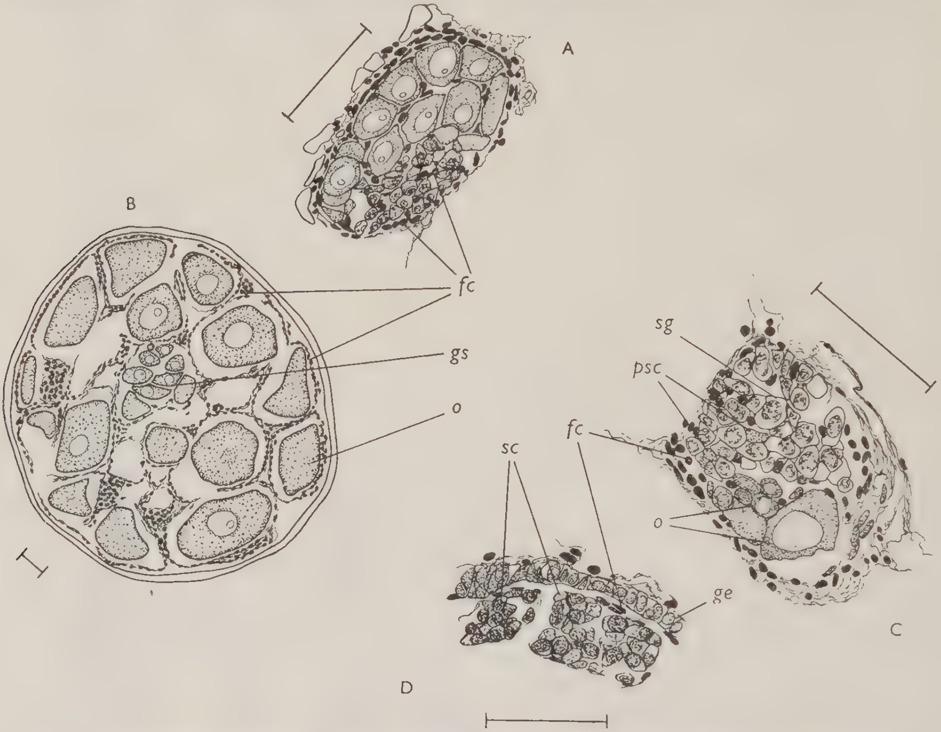


Fig. 8. Transverse sections of various gonads. A, 7-month primary female; follicle cells at oocyte interstices. B, 27-month female; a single layer of follicle cells surrounding each oocyte. C, 7-month hermaphrodite female; oocytes occupy $\frac{1}{3}$ of gonad, the spermatogonia are not grouped. D, 15-month hermaphrodite female; a small part of the periphery of the gonad showing two groups of spermatocytes close to the gonadial epithelium with follicle cells beginning to surround each group. *fc*, follicle cell; *ge*, gonadial epithelium; *gs*, germinal strand; *o*, oocyte; *psc*, 'primary sex cell'; *sc*, grouped spermatocytes; *sg*, spermatogonia. Each scale represent 0·1 mm.

In prawns 7 months old the walls of the gonad are lined with a layer of follicle cells while others surround the developing oocytes (Fig. 8). The nuclei of the follicle cells stain heavily, while the 'attenuation' of the cytoplasm makes it impossible to distinguish any cell walls. Oocytes are still being proliferated, the largest are peripheral and 0·05 mm long. The oocytes are angular with the follicle cell nuclei in the interstices. In 9-month primary females growth rate of the oocytes increases, although the general appearance of the ovary remains the same. The rate of proliferation of oocytes is maintained, the gonad increasing in diameter until the prawn is 11½ months old when the rate begins to drop. By this time the largest eggs are 0·15 mm long and small vacuoles appear in the cytoplasm. The interstitial cells show no change in appearance, but the germinal strand, while still asymmetrical, is more centrally placed. The oviduct is still a narrow tube. Acceleration of the growth rate of the oocytes continues, reaching a maximum rate in the 16-month prawn and this rate is maintained until the eggs are laid. At 16 months yolk granules appear and the oocytes are more rounded. The largest recorded oocyte diameter was 0·5 mm. This was recorded from a prawn in a sample in which most females had laid their eggs. During the last few days before laying or perhaps immediately after laying the oocyte must more than double its diameter as the smallest newly hatched eggs measure 1·1 mm (maximum diameter). The cytoplasm of the follicle cells of 16-month old females is better defined and the number of cells increases. The germinal strand remains after the eggs are laid and a number of small oocytes remain close to it. The largest remaining oocytes (0·15 mm) quickly take up a peripheral position where they are surrounded by the now very well-developed follicle cells. The latter, both round the oocytes and lining the ovary epithelium, are two or three layers thick. From the release of the mature eggs (about 18 months) until the 24th month little change occurs in the ovary. The follicle cells if anything increase slightly but the number of oocytes appears to remain constant. The remaining oocytes do not grow and show no sign of disintegration. Prior to the larvae hatching, the follicle cells decrease in size and number. The central cells are the first to return to the 'resting state', those at the periphery remain prominent until after the larvae are hatched, i.e. 25 months. At this time the remaining oocytes start to grow again and proliferation of new oocytes recommences. In 27-month-old prawns yolk is appearing in the large peripheral oocytes (0·22 mm max. diam.) and a similar cycle of events to that described follows. Maximum growth of the oocytes is reached when the prawn is 28 months old and by this time there is little proliferation of new cells, although the germinal cord surrounded by small cells remains. The maximum recorded oocyte size was 0·53 mm in a 30-month-old prawn. Egg laying commences at 30½ months. As before, only those eggs more than 0·15 mm are released, and the follicle cells again are greatly developed. The largest remaining oocytes take up a peripheral position surrounded by follicle cell layers. There is no further proliferation of oocytes and at 33 months the first sign of disintegration is seen. The prominent nucleolus is lost and the nuclear membrane is no longer clearly defined. By the 35th month the cell walls of the oocytes have broken down and the follicle cells are reduced.

As before, the latter are well developed while the eggs are carried on the pleopods.

Hermaphrodite females. All prawns showing external male characters have an ovarian germinal strand, together with a few oocytes which occupy between a quarter and a third of the area of the gonad in cross-section. The oocytes are in the 'mature' condition (Berkeley, 1930) having a clear nucleus, well-developed nucleolus and an enlarged heavily staining cytoplasm. The ovarian portion is acentric occupying a dorso-lateral position. The maximum oocyte size in the smallest prawns ($5\frac{1}{2}$–7 months old) was 0·04 mm. In these specimens, spermatogonia filled the rest of the gonad and although the cells are tightly packed there is no definite tubule structure (Fig. 8). The nuclei show the typical 'bouquet' stage of actively dividing primary and secondary spermatogonia described by Runnström (1925) with clumps of chromatin material at the periphery. Follicle cells line the gonadial walls, and typically, the oocytes, and there are a few scattered between the spermatogonia. A few cells among the latter stain heavily with the acid fuchsin of Mallory's Triple Stain and these probably correspond with the 'primary sex cells' described by Berkeley (1930). Sections show little change until after the prawns are 11 months old. By the 13th month spermatocytes are present. The gonad is larger and the oocytes still occupy about a third of the gonad. There has been some proliferation of oocytes to maintain the relative proportions and there is slight growth of the outer oocytes (0·08 mm max. diam.). Spermatogonia are still present close to the oocytes as a tight packed mass, but peripherally the cells are no longer in the 'bouquet' stage and are lighter staining than the latter. There is no obvious tubular arrangement and follicle cells are scattered between the spermatocytes. In a 14-month-old prawn the peripheral spermatocytes appear to be divided into groups. Although a few follicle nuclei are present between the groups the latter do not appear to be surrounded by the follicle cells (Fig. 8). The tenuous nature of the follicle cell's cytoplasm makes it difficult to determine this with any surety. Berkeley (1930) refers to this cytoplasm as a 'plasma without cell walls'. By 16 months the peripheral groups are clearly surrounded by follicle cells. While there are still many densely packed spermatocytes close to the oocytes, much of the testis is filled with the groups of larger spermatocytes. During the next month sperms are produced. These are of the typical four-rayed type. At the same time there is a great increase in the number of follicle cells. As yet there are no sperms in the vas deferens, but by 18 months most of the sperms have been transferred and the follicle cells surrounding the remaining spermatocytes increase further. The remaining spermatocytes are the remnants of those that were close to the oocytes. The oocytes, also surrounded by follicle cells, proliferate rapidly and the peripheral oocytes have grown to 0·15 mm (max. diam.). By $21\frac{1}{2}$ months fertilization has occurred and the immature oocytes nearly fill the gonadial cavity. The walls of the gonad are very thick and this is due in part to follicle cells and in part to the remains of what appear to be spermatocytes. A similar condition is reported by Aoto (1952) for *P. kessleri* and by Fasten (1926) in the crab *Lophopanopeus bellus*. Both these authors consider that the 'spermatocytes' have (or had) a nutritive function. As yet there is no correlated change in the external sexual characters. This does not occur until the prawn is at least 22 months old (see p. 199). During the transition of the external characters the follicle cells return to the inter-breeding state, but as much as an eighth of the gonadial space may be occupied with the remaining spermatocytes. The latter gradually disappear and few prawns older than 25 months have any trace of male cells in the gonad. The oocytes completely fill the gonad, the largest having a maximum diameter of 0·18 mm. By the 27th month the growth rate of oocytes is maximum and their future development is similar to that of primary females of the same age. It was not appreciated until sections had been examined that there was a considerable lag in the change of male external characters. Thus typical male external characters may be present in a prawn 27 months old yet the ovary may show no remains of spermatocytes, no hypertrophy of the follicle cells and oocytes proliferating rapidly. There is no change in the male external characters and no proliferation of oocytes until the follicle cells (and perhaps the remaining spermatocytes) regress. It also appears (p. 203) that there is a similar correlation between the regression of the follicle cells and the development of the second batch of oocytes in the primary females. Inhibition by the follicle cells can be postulated. In case of the hermaphrodite female this may modify the effect of a sex reversal hormone of the X organ-sinus gland.

Secondary females. Gonadial evidence of sex change in immature prawns with male external characters were found. Earliest sections were of a 9½-month-old prawn with typical male external characters. In this case the oocytes were greatly developed and occupied two-thirds of the gonad. The peripheral oocytes were twice normal size for that age (0·09 mm max. diam.). Male cells were restricted to the inner lateral side and were atypical, the spermatocytes stained very densely and the nuclei were difficult to distinguish. Follicle cells were typical and were scattered among the spermatocytes as well as among the oocytes. A second prawn of the same age showing transitional external characters had no trace of male cells in the gonad. A third prawn, 11 months, showing transition characters, had male cells restricted to an eighth of the gonad. Others examined showed a similar picture. This clear lag between change in the gonad and change in the external characters will be further discussed (p. 215).

BREEDING

One brood is produced each year, and Rasmussen (1953) has shown that temperature affects the time of egg-laying and hatching. Thus, prawns from southern Norway (average temperature approximately 7° C) start to lay eggs about the second week in October and finish laying between early November and mid-December. Hatching occurs from the first week in March to the end of April (Fig. 9). There is an over-all period of 7 months when some females can be found with eggs attached to the pleopods. (The ovigerous period—completion of egg-laying to completion of hatching—is 5 months.)

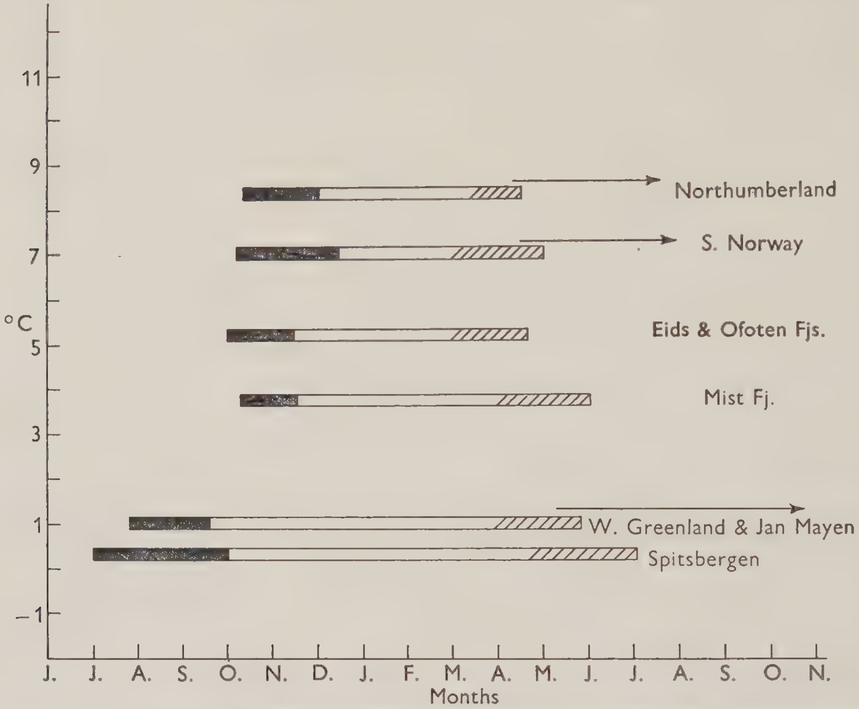


Fig. 9. Comparison of the breeding seasons of various prawn populations. Blacked section, egg-laying period; hatched section, period when larvae hatch. Arrows indicate duration of larval stages. Vertical arrangement corresponds to the mean temperature of the environment. Data extracted from Rasmussen (1953), Horsted & Smidt (1956) and the present work.

As temperature decreases so spawning starts earlier and hatching later. A maximum ovigerous period of 9 months is recorded for prawns of the Jan Mayen and Spitsbergen populations. It was expected that the Northumberland population with a high average temperature (8·5° C) would have a shorter ovigerous period. This was confirmed (Fig. 9 and Appendix II). Egg-laying commences about 10 October and all mature females are carrying eggs by 1 December. The larvae hatch between 10 March and 16 April. This represents an ovigerous period of about 4½ months (2–4 weeks less than the southern Norwegian stocks). Hatching is completed about 2 weeks earlier and the larval stages may be expected to metamorphose at least this much earlier than the Norwegian stocks.

The number of eggs varies with the size of the prawn (Fig. 10). The Northumberland population shows significant differences from the southern Norwegian stocks. The minimum total length of an ovigerous Northumberland prawn is 80 mm compared with 85 mm of the Norwegian prawns. The maximum number of eggs carried by the Northumberland prawns rarely exceeds the minimum number of eggs carried by a Norwegian prawn of the same size (Fig. 10). Thus a Northumberland prawn 85 mm total length will carry an average of 160 eggs less than a Norwegian prawn of the same length,

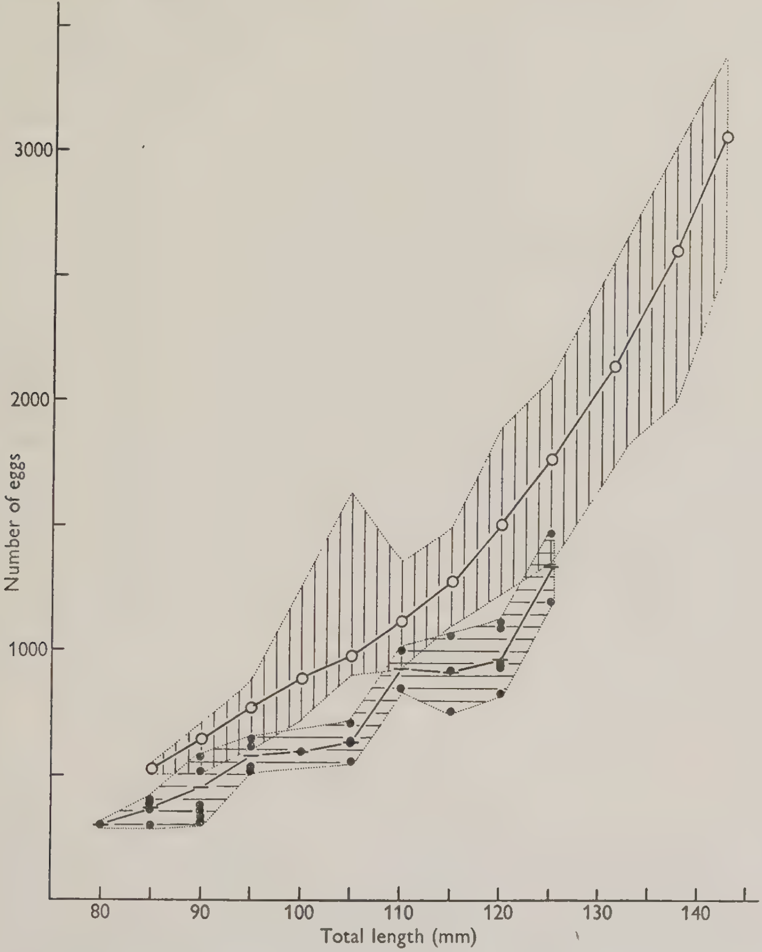


Fig. 10. Comparison of the numbers of eggs carried by *Pandalus borealis* of various lengths from Northumberland and Norwegian waters. Norwegian data (vertical hatching) extracted from Rasmussen (1953). Northumberland data (horizontal hatching). Mean numbers are indicated by the continuous line.

while the largest prawn (125 mm length) carries an average of 500 eggs less. The total number of eggs carried by the Northumberland prawn varies from 300 to 1500. Female prawns from Oslo Fjord may grow 20 mm longer than the largest Northumberland prawn and carry over 3000 eggs, i.e. the potential recruitment of the population is much less off the Northumberland coast.

Comparative figures for egg production can be calculated. Taking December–March records as a standard (i.e. at the end of egg-laying and before hatching commences) the mean number of eggs per ovigerous female for the North Sea population is 690, while those of southern Norway and West Greenland are 760 and 1300 respectively (calculated from figures given by Rasmussen, 1953; and Horsted & Smidt, 1956). These are figures for *present* productivity; an unexploited population is being compared with heavily exploited northern stocks. It is probable that the Norwegian figure, and to a lesser extent the West Greenland figure, is lower than it should be, if the populations were unexploited, for there is little doubt that fishing selects the larger prawn.

Eggs are carried on the first four pairs of pleopods. In the Northumberland population about 20% of the eggs are carried on the first pair of pleopods, 35% on both second and third pairs and 10% or less on the fourth. The lack of eggs on the fifth and the small number on the fourth allows maximum flexion of the abdomen at the junction of third and fourth abdominal segments in the backward 'escape' movement (Yonge, 1955). The packed egg volume varies from 0·35–1·76 c.c. according to size of prawn (80–125 mm total length) immediately after laying, to 0·55–2·73 c.c. prior to hatching.

In southern Norwegian stocks from 2 to 6% were found to have lost all their eggs (Rasmussen, 1953). No specimens of the Northumberland stock were found to have lost their eggs.

GROWTH RATES AND SUMMARY OF THE LIFE CYCLE

All eggs of the Northumberland population hatch by the end of the third week in April. There are no past records of the larvae being obtained in the Northumberland plankton as the identification of Jorgensen (1923) must doubtless be based on the account of Sars (1900) which Lebour (1930, 1940) shows to be of *Caridion steveni* and not *Pandalus borealis*. *Caridion steveni* occurs in Northumberland waters. Excellent descriptions of the larvae are given by Berkeley (1930). There are six stages during which time the larvae grow from 6 to 17 mm. Hjort & Ruud (1938) confirm this work from their plankton records from Oslo Fjord where metamorphosis occurs 3 months from hatching. First-stage larvae were collected from the Northumberland prawn grounds on 16 April 1957, but no other stages have been taken so far. Except for stages 1 and 2 both Berkeley and Hjort & Ruud found very few larvae in the plankton. Berkeley considers that they disappear from water close to the adult population at stage 3. Figures given by Hjort & Ruud (1938, p. 66) support this view.

The smallest post-larval prawn recorded by Hjort & Ruud (1938) was 21 mm total length (15 July 1933); but the average length of this brood on that date was 31 mm. This compares with 35 mm (2 August 1957) from Northumberland and 45 mm (October 1938) from Scottish waters (Ritchie) (Fig. 11, Appendix II). It has been established that the newly metamorphosed prawns tend to be in shallower water than the adults (Berkeley, 1930; Tåning, 1937; Hjort & Ruud, 1938; Horsted & Smidt, 1956) and these join the adult population some 9 or 10 months after hatching. In Oslo Fjord this joining of the two populations is marked by a heavy increase in the number of small prawns in catches during February–May representing between 88 and 97% of the total. Ritchie's records from Scottish waters (Appendix II) show a similar picture. There is a massive increase of young prawns in the Northumberland catches 7 months from hatching (Fig. 12). This early appearance of the new brood may be due to the small mesh size of the net, although a high growth rate and natant movement of the larvae under hydrographical conditions that tend to move them offshore may be of significance. An extensive programme of small-mesh trawling over the last 4 years in inshore waters (5–35 fathoms) has failed to catch any *P. borealis*. However, the present samples indicate that there is little mixing of the year groups, and perhaps even of sexes, for there are wide differences in the percentage composition of the various hauls (Figs. 11, 12). The young prawns of the new year-group may comprise from 20 to 80% of any sample. The average figure is 60% and this is maintained until the onset of egg-laying (Fig. 12).

Growth rate of the Northumberland population is given in Figs. 11 and 13. For the first 13 months the increased growth of the primary females over the males is slight and the average differences in total length at the end of the first year is approximately 2 mm. The average increase in length is high, 15–16 mm per month over this period. A slight drop in the rate from 7 to 8½ months coincides with low temperatures of the water during December and January before the spring increase of plankton but this may not be significant. After 13 months there is a considerable drop in the growth rate of the males which is probably related to increased testicular activity (p. 204). Between 13 and 23 months the average increase in length of the males is fairly constant at about 1·5 mm per month. There is no change at maturity. After 14 months the primary females also show a gradual falling off in their rate of growth until maturity is reached and the eggs are laid. At their first maturity the primary and secondary females are about 10 mm longer than the males. During the period when the eggs are carried (19–24 months) there is no growth, i.e. no moulting takes place while egg-bearing. From 23 to 28 months male external characters change to the female state. During this period growth rate is fairly rapid, approximately 4 mm/month. After the

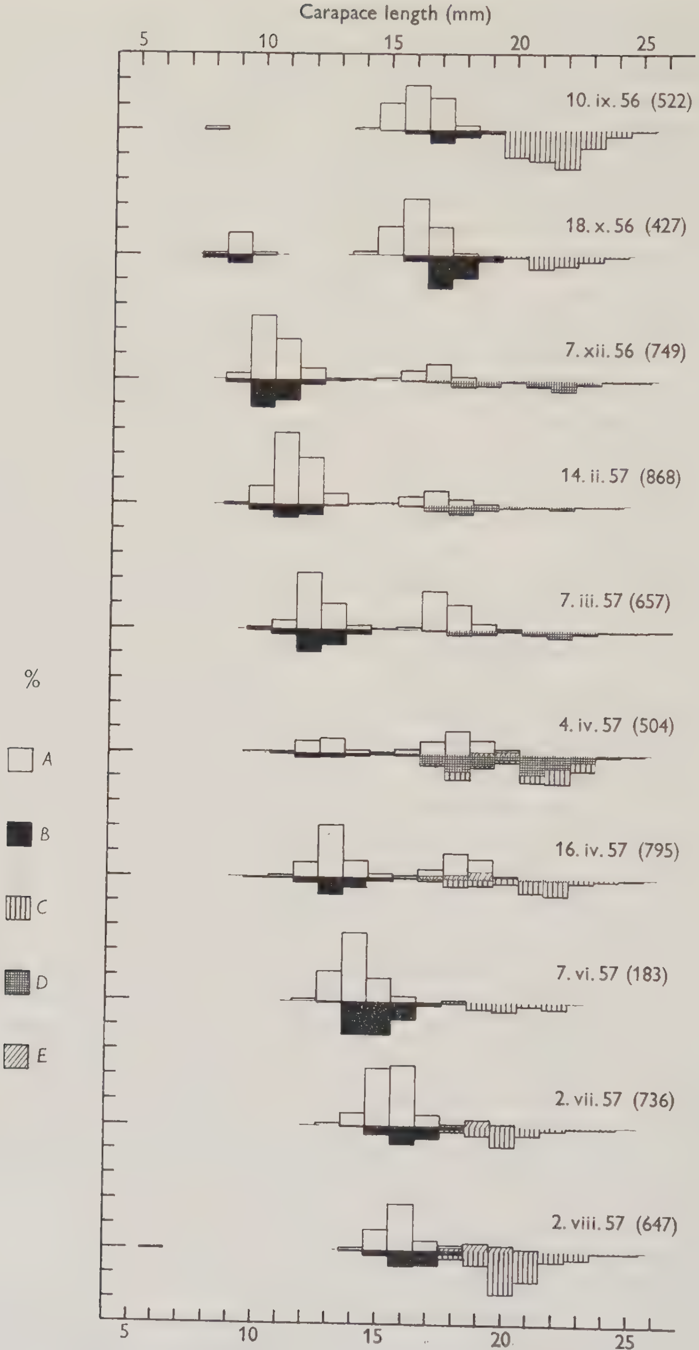


Fig. 11. Percentage size-composition of ten samples of *P. borealis* from Northumberland waters. *A*, males; *B*, immature females; *C*, females + eggs; *D*, females − eggs; *E*, transitional. The total number of prawns in each catch is given.

larvae have hatched the growth rate of the primary females is similar to the transitional males until 29 months, 2 months before the eggs are due to be laid. By this time the external characters have completely changed and the average length of a 29-month-old prawn is 108 mm. Further growth is slow and little more than 2 mm is added to the length before eggs are laid. After egg-laying no further growth occurs and the prawns die soon after the larvae hatch 4 or 5-months later. Ritchie's figures, which are a compilation of several years samples (Appendix II), show a similar picture.

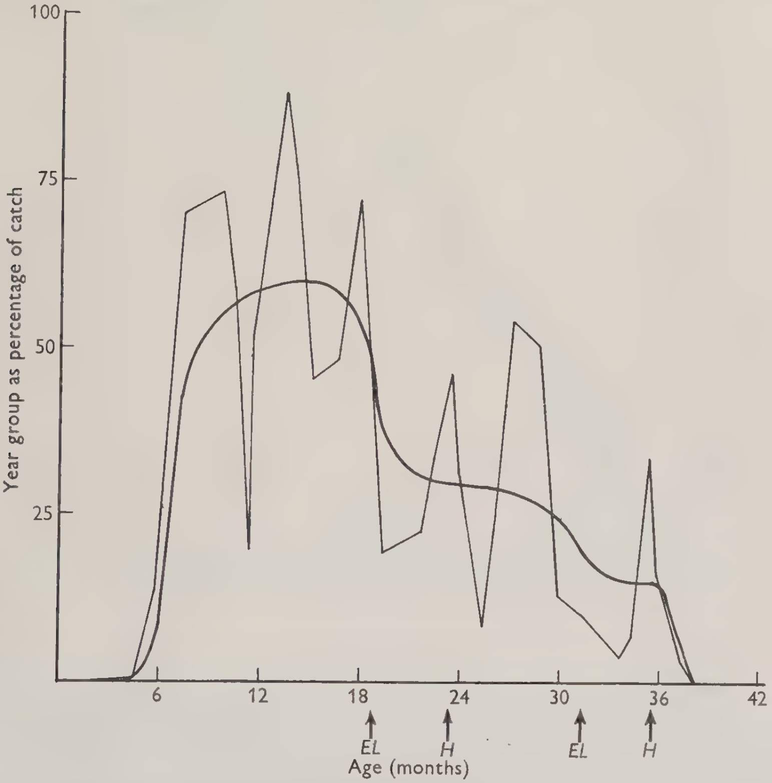


Fig. 12. Graph to show the variation in the percentage representation of one year group within the Northumberland population during the group's life. Estimated from the percentage composition of various samples. Thick line is the calculated mean. *EL*, egg-laying; *H*, hatching of larvae.

As might be expected, there is a very high mortality rate at the spawning period (Fig. 12). 50% of 18-month-old prawns die. This mortality takes place at egg-laying rather than egg-hatching and there is evidence of a second increase in the mortality rate at 31 months. Obviously this is of considerable significance for prawn fisheries, particularly as there is evidence that there is a tendency towards overfishing in Norwegian waters.

The growth rate of the Northumberland population (Fig. 14) is similar to that of southern Norway (Rasmussen, 1953). The higher temperature does not give rise to a marked increase in the growth rate. However, the maximum length attained by the Northumberland prawns (110 mm) is significantly less than that of other populations (> 120 mm). Although difficult to postulate a reason in the case of *P. borealis* this may well be a temperature effect. As a generalization, animals living at low temperatures are likely to be larger than related warm water species. It is of interest that the maximum prawn lengths of populations off the Scottish coast (Appendix II) are intermediate between Northumberland and southern Norway.

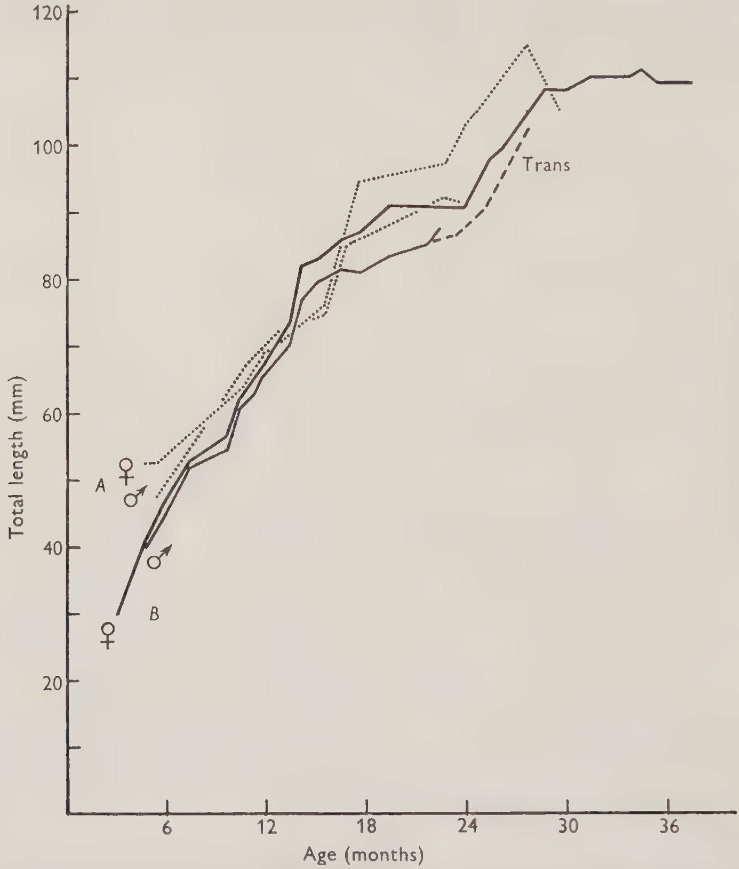


Fig. 13. Average growth-rate curves of male and female prawns from *A*, Scottish and *B*, Northumberland waters. *TRANS*, transitional Northumberland prawns.

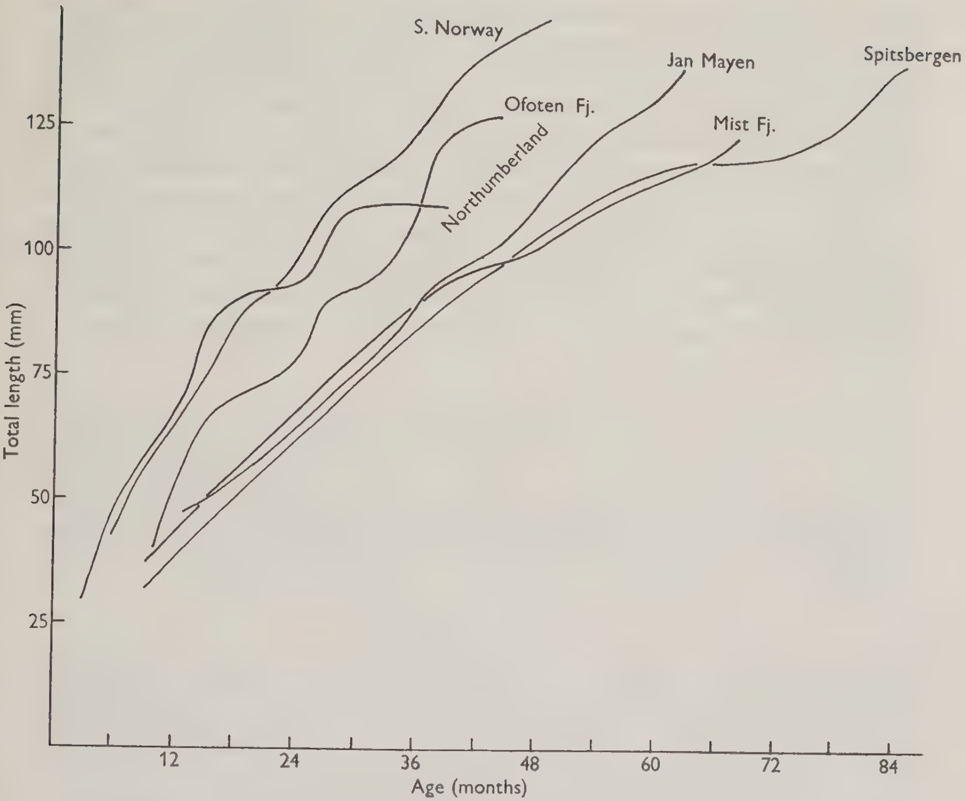


Fig. 14. Comparison of the growth rates of various prawn populations. Data from Rasmussen (1953) and present work.

MOULTS

Knowledge of the frequency of moults is incomplete. Berkeley (1930) shows that there are six larval stages. The seventh moult involves metamorphosis. Hjort & Ruud (1938) conclude that there are many moults during the first year of life until maturity, as many as fourteen in growing from 21 to 93 mm body length. Following maturity fewer moults take place. Wollebaek (1903) and Bjørk (1911) believe that large females have two moults only between each ovigerous period, although they note that 'soft' prawns are always present in their samples.

The present work gives additional information. There are at least three transitional first pleopod stages between full male and female structure. It is probable that four moults transform the male characters of the first pleopod and at least one more is required for final hypertrophy of appendix masculina. The assumption of 'breeding dress' involves a further moult. In the primary females, there is a moult immediately after the first batch of larvae hatch in which 'breeding dress' is lost and a further moult to regain it for the second egg-laying. These, presumably, are the two moults that Wollebaek (1903) and Bjørk (1911) refer to. In the Northumberland population, although the long setae are lost, the extended coxal plates of the ovigerous primary females do not return to their normal size at the first moult after the larvae hatch. They do so after a second moult that probably occurs within a month of the first, i.e. there are at least three moults between ovigerous periods. After the final egg-laying, i.e. at 30½ months, there is little or no growth. However, breeding dress is lost before death although the coxal plates remain somewhat enlarged. It seems probable that there is one moult between the hatching of the larvae and death.

PARASITES

No isopod parasites were recorded from the Northumberland population of *Pandalus borealis*, although *P. montagui* and *Spirontocaris lilljeborgii* also occuring on the same ground are affected by *Phryxus abdominalis*. The latter was parasitic on male *Pandalus borealis* of both Norwegian and West Greenland populations (Hjort & Rudd, 1938; Horsted & Smidt, 1956).

DISCUSSION

From the results of studies of prawn populations over a wide range of temperatures, a hormonal control of sex may be postulated, sex change thought of as being dependent on the concentration of an 'ovarian inhibiting substance'. Such a concept reconciles the ideas of Jägersten (1936) in which the prawns possess both male and female potentialities with the former capable of repression early in life, and of Hjort & Ruud (1938) who postulate a 'labile equilibrium' between male and female potentialities, with that of Rasmussen (1953) who links sex with size. Recently Carlisle is reported to have found that sex reversal in *P. borealis* is essentially similar to that in *Lysmata seticaudata* (*Rep. of Council of M.B.A.* 1956–7, p. 17)—'reversal is controlled primarily by action of the X organ-sinus gland complex in secreting the ovarian inhibiting substance, which appears to be the only hormone concerned. This hormone, or rather the cessation of its secretion, is responsible for the control of the onset of sex reversal. *The actual assumption of female form seems to take place at the moult following the attainment of minimum ovarian size. The degeneration of the testis which follows sex reversal* is probably to be attributed to the blocking of the opening of the vasa deferentia which *takes place when the animal develops a female shell*'.[1] While this report may fit the observed details of the biology of the Swedish prawns on which this work was based, it does not entirely fit with the present work on the Northumberland population. Although there seems little doubt that the control of the attainment of the female phase is due to lack of hormonal secretion of the X organ-sinus gland complex, it is clear that 'minimum ovarian size' is not critical, at least in southern Norway and North Sea populations. Presumably temperature must play a considerable role in sex reversal. In addition, the present study shows that testis degeneration begins before the animal develops a female shell. External changes may lag considerably behind gonadal changes and specimens found with no trace of spermatocytes may still show male external characters. Sections suggest that the state of the follicular tissue may be of importance in the change of external characters. Until the follicular tissue degenerates there is no external change. Follicular tissue is not proliferated in immature specimens so that there is no bar to the rapid change of external form. Sections also suggest that proliferated follicular tissue has an important role during the incubation of the eggs perhaps as a growth inhibitor to the prawn and the onset of the increase in the rate of proliferation of oocytes also appears to coincide with degeneration of follicular tissue.

---

[1] Author's italics.

While the X organ-sinus gland is probably all important, modification of its action may come through hormones produced within the gonad. If growth rate and gonadal development are controlled directly or indirectly by hormones, it is likely that maturity will be reached within narrow size limits, and temperature, by its general effect on metabolism (including hormone action) will influence the age of maturity and also the onset of the breeding season.

Gonadal development and histology is similar to other *Pandalus* species described by Berkeley (1930), Aoto (1952) and Mistakidis (1957).

Present data show that there can be little prospect of a fishery for this prawn in the North Sea. Ritchie's samples from Scottish waters (mostly from the Fladen area) show that the population is patchy and that numbers are not very large. The *maximum* catch off the Northumberland coast was 3 l./h using a 9 ft. beam trawl. Presumably this can be increased by using a larger trawl but cannot compare with the catches, considered very good, of 60–100 l./h from the Gulf of Maine and Norwegian waters. Even higher catches are recorded from West Greenland (Horsted & Smidt, 1957). It is difficult to extract figures from the papers referred to, but the average daily yield per boat in Norwegian waters, approximately 10 kg ≡ 18 l., could perhaps be equalled off Northumberland only under the most favourable conditions. This is likely to be the exception rather than the rule.

The ground is not extensive and is some distance from the coast. By using the commercial pink shrimp trawl, large numbers of small prawns were caught during this work and if normal British shrimp trawls were used to fish the stock it would soon be depleted. Stocks will survive if only the largest prawns (> 95 mm) are removed, fishing being limited to the period May to September when recently hatched prawns have yet to join the adult stocks.

Weather largely limits the use of small inshore boats to this period anyway, but it must be concluded that this population can only support an occasional fishery.

SUMMARY

Data on the distribution of *Pandalus borealis* have been brought up to date, and the factors controlling its distribution (temperature, salinity, substratum and depth) are discussed.

An account of the biology of the population off the Northumberland coast is given and compared with accounts of work on other populations. Knowledge of the biology of this species now extends over the entire north-south range in the eastern Atlantic.

Details of the histology and development of the gonads are given, particularly with respect to the development of the ova and to sex reversal. Sex changes in the gonad are considered in relation to changes in external form.

The growth rate of the North Sea prawn is described and compared with that of other populations.

It is unlikely that the North Sea populations can provide an additional fishery.

REFERENCES

AOTO, T., 1952. Sexual phases in the prawn, *Pandalus kessleri* Czerniavski, with special reference to reversal of sex. *J. Fac. Sci. Hokkaido Univ.*, Ser. 6 (Zool.), Vol. 2, pp. 1–20.

BELL, T., 1853. *A History of the British Stalk-eyed Crustacea.* London.

BERKELEY, A., 1930. The post-embryonic development of the common pandalids of British Columbia. *Contr. Canad. Biol.*, N.S., Vol. 6, No. 6, 85 pp.

BJØRK, W., 1911. Bidraztil kännedomen om nordhafstäkans (*Pandalus borealis* Kr.) urbredning och biologi i Kattegat om Skagerak. *Svenska hydrogr.-biol. Komm. Skr.*, No. 4 (1910–11).

BLACKER, R. W., 1957. Benthic animals as indicators of hydrographic conditions and climatic change in Svalbard waters. *Fish. Invest. Lond.*, Ser. 2, Vol. 20, No. 10, 49 pp.

CALMAN, W. T., 1899. On the British Pandalidae. *Ann. Mag. nat. Hist.*, Ser. 7, Vol. 3, pp. 27–39.

EKMAN, S., 1953. *Zoogeography of the Sea.* 417 pp. London.

FASTEN, N., 1926. Spermatogenesis of the black-clawed crab, *Lophopanopeus bellus* (Stimpson) Rathbun. *Biol. Bull., Woods Hole*, Vol. 50, pp. 277–93.

GORBUNOW, G., 1934. Die Decapoden Crustaceen der nördlichen Hälfte des Karischen Meeres. *Trans. arct. Inst., Leningr.*, Vol. 9, pp. 67–77.

GRIEG, J. A., 1925. Evertebrata fra bankerne ved Spitsbergen (Fiskenaering og bundfauna). *Bergens Mus. Aarb.*, No. 9, 33 pp.

—— 1926*a*. Evertebrata fra bankerne ved Spitsbergen. *Bergens Mus. Aarb.*, No. 5, 28 pp.

—— 1926*b*. Decapoda Crustacea from the West Coast of Norway and in the North Atlantic. *Bergens Mus. Aarb*, No. 7, 53 pp.

—— 1932. Decapoda Crustacea fra bankerne ved Svalberg. *Bergens Mus. Aarb.* No. 2, 34 pp.

HEEGAARD, P. E., 1941. The Zoology of East Greenland. Decapod crustaceans. *Medd. Grønland*, Bd. 121, No. 6.

HJORT, J. & RUUD, J. T., 1938. Deep-sea prawn fisheries and their problems. *Hvalråd. Skr.*, No. 17, 144 pp.

HOFSTEN, N., 1916. Die Decapoden Crustaceen des Eisfjordes. *Svenska VetenskAkad. Handl.* Bd. 54, No. 7, 108 pp.

HOGLUND, H., 1943. On the biology and larval development of *Leander squilla* (L) Forma *typica* de Man. *Svenska hydrogr.-biol. Komm. Skr.*, N.S. (Biologi), Bd. 2, No. 6, 44 pp.

HORSTED, S. A., & SMIDT, E., 1956. The deep sea prawn (*Pandalus borealis* Kr.) in Greenland waters. *Medd. Komm. Havundersøg., Kbn.*, N.S., Bd. 1, No. 11, 118 pp.

HYNES, F. W., 1929. Shrimp fishery of South-east Alaska. *Rep. U.S. Comm. Fish.*, Year 1921, Appendix 1, 18 pp. (Bureau of Fisheries, Doc. No. 1052.)

JÄGERSTEN, G., 1936. Über die Geschlechtsverhältnisse und das Wachstum bei *Pandalus. Ark. Zool.*, Bd. 28, A, No. 20, 26 pp.

JOHNSON, F. F. & LINDNER, M. J., 1934. Shrimp industry of the South Atlantic and Gulf States. *Invest. Rep. U.S. Bur. Fish.*, No. 21.

JORGENSEN, O. M., 1923. Plankton investigations, 1921–22. III. Marine plankton (4) Crustacea. *Rep. Dove mar. Lab.*, N.S., Vol. 12, pp. 112–33.

KUBO, I., 1951. Bionomics of the prawn, *Pandalus kessleri* Czerniavski. *J. Tokyo Univ. Fish.*, Vol. 38, No. 1, 26 pp.

LEBOUR, M. V., 1930. The larval stages of *Caridion*, with a description of a new species, *C. steveni. Proc. zool. Soc. Lond.*, 1930, pp. 181–94.

—— 1940. The larvae of the Pandalidae. *J. mar. biol. Ass. U.K.*, Vol. 24, pp. 239–52.

LEOPOLDSEDER, F. 1934. Geschlechtsverhältnisse und Heterochromosomen bei *Pandalus borealis* Kröyer (Decapoda). *Z. wiss. Zool.*, Bd. 145, pp. 337–50.

MISTAKIDIS, M. N., 1957. The biology of *Pandalus montagui* Leach. *Fish. Invest., Lond.*, Ser 2, Vol. 21, No. 4, 52 pp.

NORMAN, A. M. & BRADY, G. S., 1909. The Crustacea of Northumberland and Durham. *Trans. nat. Hist. Soc. Northumb. Durh.*, N.S., Vol. 3, pp. 252–417.

PIKE, R. B., 1952. Notes on the biology of the prawn *Pandalus bonnieri* Caullery. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 259–67.

POULSEN, E. M., 1946. Investigations on the Danish fishery for and the biology of the Norway lobster and the Deep Sea Prawn. *Rep. Danish biol. Sta.*, Vol. 48, pp. 27–57.

PRUTER, A. T. & HARRY, G. Y., 1952. Results of preliminary shrimp explorations off the Oregon coast. *Fish. Comm. Res. Briefs*, Vol. 4, No. 1.

RASMUSSEN, B., 1942. Om dypvamisreken ved Spitsbergen. *Rep. Norweg. Fish. Invest.*, Vol. 7, No. 4, 43 pp.

——1949. Problems in the fishery for Deep Sea Prawns. *Rapp. Cons. Explor. Mer*, Vol. 128, pt. II, pp. 79–81.

—— 1953. On the geographical variation in growth and sexual development of the Deep Sea Prawn. *Rep. Norweg. Fish. Invest.*, Vol. 10, No. 3, 160 pp.

RATHBUN, M. J., 1904. Decapod crustaceans of the North-west coast of North America. *Harriman Alaska Ser.*, Vol. 10, 210 pp.

—— 1929. Canadian Atlantic Fauna, 10. Arthropoda. *Publ. biol. Bd Canada.* The Atlantic Biol. Sta., St Andrews, N.B. 38 pp.

REES, C. B., 1952. Continuous plankton records: the decapod larvae in the North Sea, 1947–49. *Hull Bull. mar. Ecol*, Vol. 3, pp. 157–84.

—— 1955. Continuous plankton records: the decapod larvae in the North Sea, 1950–51. *Hull Bull. mar. Ecol.*, Vol. 4, pp. 69–80.

RUNNSTRÖM, S., 1925. Beitrag zur Kenntnis einiger hermaphroditischen, dekapoden Crustaceen. *Bergens Mus. Skr.*, N.F., Bd. 3, pp. 1–115.

SARS, G. O., 1900. Account of the post embryonal development of *Pandalus borealis*. *Rep. Norweg. Fish. Invest.*, Vol. 1, No. 3, 45 pp.

SCHOTT, G., 1942. *Geographie des Atlantischen Ozeans*. Hamburg: Boysen.

SIVERTSEN, E., 1932. Crustacean Decapoda and Mysidacea from the East Siberia and Chukotsk Seas. *Sci. Res. Norweg. N. Polar Exped. 'Maud'*, 1918–1925, Vol. 5, No. 13.

STEVENSON, K., 1935. The Godthåb Expedition, 1928. Crustacea, Decapoda. *Medd. Grønland*, Bd. 80, No. 1.

TÅNING, Å. V., 1937. Report from North-western area committee, 1936. *Rapp. Cons. Explor. Mer*, Vol. 105, pt. II, pp. 13–19.

WEDEMEYER, H., 1912. Die Carididen der Nordsee. *Wiss. Meeresuntersuch.*, Bd. 15, pp. 105–63.

WOLLEBAEK, A., 1903. Raeker og Raekefiske. *Aarsber. Norg. Fisk.*, No. 2.

YONGE, C. M., 1955. Egg attachment in *Crangon vulgaris* and other Caridea. *Proc. roy. Soc. Edinb.*, B. Vol. 65, pt. 3, pp. 369–400.

APPENDIX I

Records of Pandalus borealis *from British Waters*

	Date	Position	Depth (m)	No. of specimens
J. A. Grieg	1904	57° 9′ N.–1° 30′ W.	96	?
R. A. Todd	26. viii. 07	55° 31½′ N–0° 33′ W.	90	30
H. Wedemeyer	1912	54° 24′ N–2° 59′ E	40	1
H. O. Bull	8. ii. 33	12 miles E. of Coquet	?	6
	28. viiii. 33	Off Coquet	?	Hundreds
	27. i. 34	'N.E. Bank'	?	1
	30. i. 34	Off Coquet	?	3
	10. iii. 34	Off Coquet	?	1
	20. ix. 34	17 miles E. of Tyne	?	Hundreds
	29. i. 35	Off Newbiggin	?	3
	10. ii. 35	Off Newbiggin	?	3
	24. ix. 35	14 miles E.S.E. Tyne	?	Many
A. Ritchie	7. iv. 36	58° 05′ N.–0° 31′ E.	150–152	81
	8. iv. 46	58° 27′ N.–0° 01′ W.	145–147	59
	26. iv. 36	58° 30′ N.–0° 24′ W.	140	1
	7. iv. 36	58° 05′ N.–0° 05′ W.	128–133	1
	13. viii. 36	58° 07′ N.–0° 52′ E.	150	114
	13. viii. 36	58° 32′ N.–0° 56′ E.	140	32
	19. ix. 36	58° 21′ N.–0° 04′ W.	134	6
	9. iii. 37	58° 05′ N.–0° 31′ E.	149	170
	24. iii. 37	58° 05′ N.–0° 25′ W.	135	16
	17. viii. 37	58° 02′ N.–0° 30′ E.	145	51
	24. iii. 38	58° 05′ N.–0° 05′ W.	140	26
	2. v. 38	57° 47′ N.–0° 40′ E.	127	48
	14. v. 38	60° 06′ N.–0° 10′ E.	121	8
	9. v. 38	58° 50′ N.–0° 26′ E.	145	6
	9. x. 38	55° 32′ N.–0° 55′ W.	98	196
	16. iii. 39	58° 01′ N.–0° 31′ E.	145	104
	18. ii. 39	59° 01′ N.–0° 25′ E.	140	12
	26. iv. 39	58° 05′ N.–0° 05′ W.	147	156
	13. iv. 39	57° 47′ N.–0° 40′ E.	130	2
	23. viiii. 39	58° 55′ N.–0° 25′ W.	132	18
J. A. Allen	1955–58	13 miles E. of Blyth	90	Hundreds
British Museum (Nat. Hist.)	6. xi. 07	55° 31½′ N.–0° 53′ W.	100	12
	16. x. 35	14 miles E.S.E. Tyne	80	10

APPENDIX II

Scottish samples 1936–39

Collected by Dr A. Ritchie, Scottish Home Department Laboratory, Aberdeen.

(Young prawns, classed as 'immature' by Dr Ritchie, have been assumed to be immature females on the grounds that (i) males of a similar size range are recorded from the same samples, and (2) the present work shows that young prawns of the same size from Northumberland can be sexed.)

Total length (mm)	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total
45	—	—	—	—	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—	—	—	—	—
55	13	—	—	—	—	13	—	3	1	—	—	4
60	20	—	2	—	—	22	14	4	5	—	—	23
65	36	—	4	—	—	40	26	4	13	—	—	43
70	16	—	5	—	—	21	20	3	8	—	—	31
75	3	1	1	1	—	6	8	3	1	—	—	12
80	2	—	—	3	—	5	1	—	—	—	—	1
85	8	7	—	10	—	25	3	2	—	—	1	6
90	19	19	—	14	1	53	13	8	—	—	3	24
95	19	35	—	4	—	58	7	22	—	—	5	34
100	4	14	—	11	1	30	—	48	—	—	20	68
105	2	13	—	13	1	29	—	17	—	—	11	27
110	—	9	—	8	2	19	—	17	—	1	4	22
115	—	2	—	2	1	5	—	—	—	—	4	4
120	—	—	—	—	1	1	—	—	—	—	1	1
125	—	—	—	—	—	—	—	—	—	—	—	—
130	—	—	—	—	—	—	—	—	—	—	—	—
135	—	—	—	—	—	—	—	—	—	—	—	—
140	—	—	—	—	1	1	—	—	—	—	—	—
T	142	100	12	66	8	328	92	131	28	1	49	301
Date	March 1937 '38 '39						April 1936 '39					

Total length (mm)	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total
45	—	—	—	—	—	—	—	—	—	—	—	—
50	—	—	—	—	—	—	—	—	—	—	—	—
55	1	—	1	—	—	2	—	—	—	—	—	—
60	8	—	5	—	—	13	—	—	—	—	—	—
65	16	—	1	—	—	17	1	—	—	—	—	1
70	13	—	5	—	—	18	2	—	1	—	—	3
75	6	—	1	—	—	7	14	—	3	—	—	17
80	2	—	—	—	—	2	27	—	8	—	—	35
85	—	—	—	—	—	—	35	1	13	—	—	49
90	—	—	—	—	—	—	15	—	11	—	—	26
95	—	—	—	—	1	1	5	2	4	—	4	15
100	—	—	—	—	—	—	1	4	—	—	15	20
105	—	—	—	—	1	1	5	—	—	—	17	22
110	—	—	—	—	—	—	—	—	—	—	8	8
115	—	—	—	—	1	1	—	—	—	—	11	11
120	—	—	—	—	—	—	—	—	—	—	8	8
125	—	—	—	—	—	—	—	—	—	—	—	—
130	—	—	—	—	—	—	—	—	—	—	—	—
135	—	—	—	—	—	—	—	—	—	—	—	—
140	—	—	—	—	—	—	—	—	—	—	—	—
T	46	—	13	—	3	62	105	7	40	—	63	215
Date	May 1939						August 1936 '37 '39					

Total length (mm)	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total
45	—	—	—	—	—	—	1	—	1	—	—	2
50	1	—	—	—	—	1	4	—	1	—	—	5
55	1	—	—	—	—	1	7	—	—	—	—	7
60	—	—	—	—	—	—	1	—	—	—	—	1
65	—	—	—	—	—	—	—	—	—	—	—	—
70	—	—	—	—	—	—	—	—	—	—	—	—
75	—	—	—	—	—	—	2	—	—	—	—	2
80	—	—	—	—	—	—	9	—	—	—	—	9
85	1	—	—	—	—	1	35	—	—	—	6	41
90	—	—	—	—	—	—	15	—	—	—	25	40
95	—	—	—	—	1	1	2	—	—	—	29	31
100	—	—	—	—	1	1	1	—	—	—	26	27
105	—	—	—	—	1	1	1	—	—	1	13	15
110	—	—	—	—	—	—	—	—	—	—	11	11
115	—	—	—	—	—	—	—	—	—	—	4	4
120	—	—	—	—	—	—	—	—	—	—	1	1
125	—	—	—	—	—	—	—	—	—	—	—	—
130	—	—	—	—	—	—	—	—	—	—	—	—
135	—	—	—	—	—	—	—	—	—	—	—	—
140	—	—	—	—	—	—	—	—	—	—	—	—
T	3	—	—	—	3	6	78	—	2	1	118	196
Date	September 1936						October 1938					

APPENDIX II

Northumberland samples, 1956–57

Carapace length (mm)	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	1	—	2	—	—	3	6	—	2	—	—	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	35	—	12	—	—	47	21	—	11	—	—	32	3	—	4	—	—	7	—	—	—	—	—	—
10	—	—	—	—	—	—	3	—	2	—	—	5	178	—	79	—	—	257	61	—	26	—	—	87	5	—	1	—	—	6
11	—	—	—	—	—	—	—	—	—	—	—	—	121	—	63	—	—	184	228	—	72	—	—	300	22	—	15	—	—	37
12	—	—	—	—	—	—	—	—	—	—	—	—	33	—	15	—	—	48	160	—	39	—	—	199	148	—	66	—	—	214
13	—	—	—	—	—	—	—	—	—	—	—	—	2	—	1	—	—	3	36	—	5	—	—	41	65	—	42	—	—	107
14	2	—	—	—	—	2	6	—	—	—	—	6	1	—	—	—	—	1	2	—	1	—	—	3	10	—	14	—	—	24
15	29	—	2	—	—	31	47	—	—	—	—	47	5	—	—	—	—	5	4	—	—	—	—	4	1	—	—	—	—	1
16	92	—	9	—	—	101	93	—	12	—	—	105	29	—	—	2	—	31	29	—	—	3	—	32	7	—	—	—	—	7
17	63	—	28	—	—	91	50	—	58	—	—	108	51	—	—	6	—	57	53	—	—	15	—	68	92	—	—	10	—	102
18	7	—	16	—	—	23	4	—	30	—	—	34	10	—	—	22	—	32	24	—	—	37	—	61	66	—	—	15	—	81
19	—	—	4	—	5	9	—	—	10	—	—	10	—	—	—	18	—	18	1	—	—	18	—	19	18	—	—	10	—	28
20	1	—	—	—	61	62	—	—	1	—	2	3	—	—	—	6	—	6	—	—	—	14	—	14	2	—	—	3	—	5
21	—	—	—	—	65	65	—	—	—	—	24	24	—	—	—	21	—	21	—	—	—	11	—	11	—	—	—	13	—	13
22	—	—	—	—	81	81	—	—	—	—	20	20	—	—	—	34	—	34	—	—	—	16	—	16	—	—	—	19	—	19
23	—	—	—	—	39	39	—	—	—	—	9	9	—	—	—	13	—	13	—	—	—	3	—	3	—	—	—	8	—	8
24	—	—	—	—	13	13	—	—	—	—	1	1	—	—	—	5	—	5	—	—	—	3	—	3	—	—	—	2	—	2
25	—	—	—	—	3	3	—	—	—	—	—	—	—	—	—	2	—	2	—	—	—		—	—	—	—	—	2	—	2
26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—	1	—	1
T	195	—	61	—	266	522	244	—	127	—	56	427	541	—	169	129	—	749	601	—	147	120	—	868	436	—	138	83	—	657
Date	10 September 1956						18 October 1956						7 December 1956						14 February 1957						7 March 1957					

Carapace length (mm)	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total	♂	Trans	Immature ♀	♀+Eggs	♀−Eggs	Total
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	2	—	—	—	—	2	—	—	2	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	2	—	3	—	—	5	2	—	4	—	—	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	25	—	11	—	—	36	49	—	20	—	—	69	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
13	30	—	10	—	—	41	165	—	58	—	—	223	22	—	1	—	—	23	3	—	—	—	—	3	—	—	—	—	—	—
14	7	—	8	—	—	15	52	—	33	—	—	85	50	—	25	—	—	75	36	—	5	—	—	41	4	—	1	—	—	5
15	1	—	1	—	—	2	12	—	12	—	—	24	17	—	25	—	—	42	169	—	29	—	—	198	53	—	16	—	—	69
16	9	—	—	1	1	11	7	—	1	1	3	12	4	—	14	—	—	18	176	—	58	—	—	234	90	—	44	—	—	134
17	25	—	—	17	8	50	23	3	—	1	11	38	—	—	3	—	—	3	30	—	39	—	—	69	25	—	42	—	—	67
18	46	—	—	37	17	100	73	7	—	2	24	106	—	1	—	—	1	2	3	3	8	—	7	21	7	6	11	—	11	35
19	27	1	—	26	3	57	40	19	—	1	24	84	—	—	—	—	5	5	—	6	—	—	29	35	—	20	—	—	39	59
20	5	4	—	13	5	27	7	4	—	—	12	23	—	—	—	—	6	6	—	21	—	—	63	84	—	13	—	—	119	132
21	—	—	—	41	17	58	—	—	—	4	42	46	—	—	—	—	3	3	—	9	—	—	28	37	—	2	—	—	83	85
22	—	—	—	30	30	60	—	—	—	4	50	54	—	—	—	—	5	5	—	—	—	—	9	9	—	—	—	—	30	30
23	—	—	—	18	17	35	—	—	—	—	15	15	—	—	—	—	—	—	—	—	—	—	4	4	—	—	—	—	23	23
24	—	—	—	4	1	5	—	—	—	—	7	7	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	4	4
25	—	—	—	—	1	1	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	4
26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
T	179	5	33	187	100	504	430	33	130	13	189	795	94	1	68	—	20	183	417	39	139	—	141	736	179	41	114	—	313	647
Date	4 April 1957						16 April 1957						7 June 1957						2 July 1957						2 August 1957					

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

ALLEN, J. A., 1958. On the basic form and adaptations to habitat in the Lucinacea (Eulamellibranchia). *Phil. Trans.* B, Vol. 241, pp. 421–84.

The functional morphology of the Lucinacea is described. In particular, comparative accounts of the feeding, respiratory and cleansing currents are given. All species construct an anterior inhalant tube from particles of the substratum cemented together by the secretion of the glands in the tip of the modified vermiform foot. Particles entering by this tube are sorted by the ciliated epithelium covering the elongate anterior adductor muscle. An evolutionary sequence can be demonstrated showing that as the size and efficiency of the anterior adductor increases there is a related decrease in the size and efficiency of the gills and palps and the sorting mechanisms on them. Progressive reduction in sorting mechanisms of the stomach and in numbers of apertures to the digestive diverticula is demonstrated. This simplification emphasizes the essential mechanisms involved in the functioning of the eulamellibranch stomach.

J. A. A.

ATKINS, W. R. G., 1957. The direct estimation of ammonia in sea water, with notes on nitrate, copper, zinc, and sugars. *J. Cons. int. Explor. Mer*, Vol. 22, pp. 271–7.

A method for estimating ammonia not involving distillation appears to be provided by the use of chloramine T and a pyridine pyrazolone reagent devised by J. Kruse and M. G. Mellon in 1952. A purple colour is produced and can be extracted with carbon tetrachloride and matched or measured in a spectrophotometer at 450 mμ. Even with a 1 cm cell as little as 0·025 parts per million can be determined. Other constituents of sea water do not interfere. The advantages of the diphenyl benzidine reagent for nitrates are stressed, as are also those of carbon tetrachloride for extracting copper diethyl-dithiocarbamate. For zinc the dithiocarbamate is preferable to ferrocyanide but *p*-dimethyl-aminostyryl-β-naphthiazole methiodide is better. Sugars, as arabinose, may be estimated in sea water and unialgal cultures by the use of *n*-ethyl carbazole recommended by Collier, Ray and Magnitzky.

W. R. G. A.

ATKINS, W. R. G. & POOLE, H. H., 1958. Cube photometer measurements of the angular distribution of submarine daylight and the total submarine illumination. *J. Cons. int. Explor. Mer*, Vol. 23, pp. 327–36.

The average obliquity of submarine daylight in the English Channel was again found to be between 30° and 40°. It varied little, if at all, with depth below surface, or with surface or daylight conditions, hence the ratio of the total to the vertical illumination in water never differed much from 1·25. In air it is unlikely to be less than 1·5, and may run up to 5 or 6 with low sun.

Accordingly, the ratio (total illumination in water)/(total illumination in air) is equal to the corresponding ratio for the vertical illuminations multiplied by a factor which may vary from about 1·25/1·5 for high sunlight to about 1·25/6 for low sunlight, approximately a fourfold variation.

The upward light in clear water is about 2% of the downward, but may rise to

3·5 % or more nearer shore. As before green light penetrated best; for though in pure water extinction is a minimum for blue, the effects of scattering and more especially of the absorption of plankton chlorophyll and of yellow pigments cuts down the transmission relatively heavily in the blue. W.R.G.A.

BIDDER, A. M., 1957. Evidence for an absorptive function in the 'Liver' of *Octopus vulgaris* Lam. *Pubbl. Staz. zool. Napoli*, Vol. 29, pp. 139–50.

A number of specimens of *Octopus vulgaris* were fed with crabs, previously injected with a dense suspension of carmine. The carmine was traced into the caecum, and thence, chiefly in solution, into the 'liver'. After entering the liver cells, the carmine becomes invisible, and can remain thus in the body for at least 3 days, being eliminated periodically with excretory granules contained in excretory vacuoles formed in the liver cells. The liver is shown to have three functions which each cell performs in turn: enzyme-secretion, absorption, excretion. Carmine also penetrates the liver of *Sepia officinalis*. The results are compared with those previously described for *Loligo vulgaris*, in which the liver has no absorptive function. A.M.B.

BODEN, B. P. & KAMPA, E. M., 1958. Lumière, bioluminescence et migrations de la couche diffusante profonde en Méditerranée occidentale. *Vie et Milieu*, Tome IX, 10 pp.

The presence of a migratory sonic-scattering layer in the Golfe du Lion was established. This layer was found to be established with the 5×10^{-3} μwatt/cm^2 isolume during its twilight migration toward the surface.

As the layer approached the surface, during its period of most rapid migration it appeared to overtake this isolume. Numerous bright flashes of bioluminescence were recorded at this time, and these may have obscured the true relationship between the depth of the scattering layer and the amount of transmitted light. The possibility that scattering-layer organisms possess discriminatory mechanisms which enable them to distinguish components of ambient light is discussed.

The layer did not rise above a depth of about 48 m. A sharp thermocline was present at this depth and it is suggested that this may have been a barrier to further migration. B.P.B.

CALDWELL, P. C., 1958. Studies on the internal pH of large muscle and nerve fibres. *J. Physiol.*, Vol. 142, pp. 22–62.

An investigation of the internal pH of the muscle fibres of *Carcinus maenas* and *Maia squinado* and of the giant axons of *Loligo forbesi* is described. The internal pH was measured with micro-glass pH electrodes. Normally it is near 7. When the muscle fibres and the axons were immersed in saline or sea water saturated with CO_2 it fell to values in the region of 6, but in the other bathing solutions used it changed more slowly, usually by a smaller amount. During contractures of *Maia squinado* muscle fibres induced with 0·6 M-KCl no changes in pH greater than the limits of sensitivity of the method of measurement (about 0·1 of a pH unit) could be detected. The changes in squid axons after depolarization with 0·6 M-KCl were also less than the limits of sensitivity of the method.

The results indicate that the internal pH is regulated mainly by the external CO_2 tension and possibly also by some process which brings about the active extrusion of H ions. It does not appear to be regulated by the Donnan equilibrium. P.C.C.

Cambridge, G. W., 1958. Responses of the anterior retractor muscle of the byssus of *Mytilus edulis*. *Nature, Lond.*, Vol. 182, p. 35.

A preliminary investigation of some agents which render the anterior retractor muscle of the byssus (ARMB) of *Mytilus edulis* insensitive to electrical stimulation and to acetylcholine (ACh) is reported.

Contrary to the findings of Schild using vertebrate smooth muscle, after treatment with 0·56 M-KCl the ARMB does not respond to either electrical stimulation or to ACh. The contraction produced by KCl is abolished by previous treatment with propylene glycol monophenyl ether. Both this and the KCl effect are reversible on washing with sea water. The toxin of *Gymnodinium veneficum* does not prevent the response of the muscle to d.c. stimulation. G.W.C.

Carlisle, D. B., 1958. A crustacean chromactivator. *Nature, Lond.*, Vol. 182, pp. 33–4.

Chromactivating substance A which is the stored form of one of the main colour change hormones of Crustacea is not present as such in extracts of the ganglionic X organ whose neurones appear to be the centre of production. If these extracts, however, are treated with any reagent which breaks hydrogen bonds substance A appears in large amounts. It is probably present in the form of an inactive precursor in the untreated extracts. D.B.C.

Cooper, L. H. N., 1958. Consumption of nutrient salts in the English Channel as a means of measuring production. *Rapp. Proc. Verb. Cons. Int. Explor. Mer*, Vol. 144, pp. 35–7.

Evidence is produced that this powerful method of estimating minimum productivity of the English Channel has served its turn and other methods are needed for further advance. A cruise in January 1947, designed to test the uniformity of distribution of phosphate in the Western English Channel, revealed areas with phosphate contents varying by as much as 1·7:1. Moreover, the waters poorest in nutrients had been most productive of animals. The apparent winter maximum of phosphate in 1950 occurred a month late—in February. There is evidence for the view that this apparent maximum occurred in a narrow belt of coastal water created by a storm. The figure to represent the winter maximum for a wide area needs to be a lower one observed in January and March. A process of partition of nutrients consequent upon the prevailing winds is described. L.H.N.C.

Green, J., 1958. *Dactylopusioides macrolabris* (Claus) (Copepoda; Harpacticoida) and its frond-mining nauplius. *Proc. zool. Soc. Lond.*, Vol. 131, pp. 49–54.

Nauplii and an associated adult female of *Dactylopusioides macrolabris* were found in mines between the two epidermal layers of the brown alga *Dictyota dichotoma* at Torquay, Devon. This is the first British record of this copepod, and the first record of a copepod mining the fronds of a member of the Phaeophyceae.

The second antenna of the nauplius is modified to form a biting appendage, while the mandibles are not capable of biting. J.G.

HEDLEY, R. H., 1958. A contribution to the biology and cytology of *Haliphysema* (Foraminifera). *Proc. zool. Soc. Lond.*, Vol. 130, pp. 569–76.

The grey-white, arenaceous and monothalamous foraminifer *Haliphysema tumanowiczii* is very common near Penlee Point in the Plymouth area where it can be found firmly attached, by means of a basal disc, to the fronds of the red weed *Delesseria sanguinea.*

The test, usually 1–2 mm long and exceptionally 5 mm, is composed mainly of sponge spicules, detritus and quartz grains cemented together by a mucopolysaccharide. Not many attached arenaceous foraminifera grow away from the substratum to the same extent as does *H. tumanowiczii* and this may be correlated with the presence of an organic sheath secreted by the animal on the inside of the test wall in the basal disc and first quarter of the pedicle. This is a flexible and fibrous component, composed of a mucoprotein, with the longitudinal axes of the fibres parallel to the major axis of the test. Because of the mechanical buffer action of the organic sheath the animal is able to withstand the relatively rough conditions of the littoral zone. At times an unattached spheroidal individual is released from the attached animal. These individuals were seen to move over the substratum, settle, form basal discs and eventually to grow, becoming typical attached individuals. In the opinion of the author the following forms are probably synonyms of *H. tumonawiczii* Bowerbank: *H. primordiale* Haeckel, *H. echinoides* Haeckle, *H. globigerina* Haeckel and *H. advena* Cushman.

R.H.H.

JONES, W. CLIFFORD, 1958. The effect of reversing the internal water-current on the spicule orientation in *Leucosolenia variabilis* and *L. complicata. Quart. J. micr. Sci.*, Vol. 99, pp. 263–78.

Oscular tubes of *Leucosolenia* were excised and tied on the ends of fine glass tubes through which a current of sea water was allowed to siphon. The tubes were mounted either at the basal or at the oscular end, with or without the original oscular rim. Spicules subsequently developing in the wall tended to be oriented towards the distal end in the distal part of the tube and towards the mounted end in the proximal part, a confused zone lying in between. The orientation was but little affected by the removal of the original oscular rim, and only coincided with the direction of the internal water-current over part of the tube. The orientation cannot, furthermore, depend on static structural features in the wall. A mechanical hypothesis explaining the orientation is briefly described.

W.C.J.

SOUTHWARD, A. J. & SOUTHWARD, E. C., 1958. Pogonophora from the Atlantic. *Nature, Lond.*, Vol. 181, p. 1607.

SOUTHWARD, A. J., 1958. Abundance of Pogonophora. *Nature, Lond.*, Vol. 182, p. 272.

Several species of *Siboglinum*, including some new species, have been dredged up from the continental slope between lat. 47° 56′ N., long. 7° 56′ W. and lat. 48° 32′ N., long. 10° 11′ W., at depths of 300 to 710 fm. Specimens of the same genus have also been found in bottom samples taken by Mr N. A. Holme in 80–90 fm. off Dingle Bay, Ireland. The Pogonophora now appear to be among the commonest animals from muddy bottoms, and not only in deep water. Previous recognition may have been hindered by the resemblance of the tubes to fibres used in dredge and trawl netting.

A.J.S.

J. mar. biol. Ass. U.K. (1959) 38, 225–238
Printed in Great Britain

THE HABITS AND FEEDING ORGANS OF *DENTALIUM ENTALIS*

By J. E. Morton
Department of Zoology, Queen Mary College, University of London

(Text-figs. 1–5)

The Scaphopoda or tusk-shells are the smallest and most uniform class of molluscs. They burrow in sand of medium to coarse shelly grade, and specialize on a diet of hard-shelled microbenthos, particularly Foraminifera. *Dentalium entalis* L. is the commonest West European species, and may become locally dominant. Le Danois (1948) has described a 'Dentalietum' in deeper water sandy ground at about 100 fathoms in the southern Celtic Sea. Stephen (1933) discusses the distribution of *D. entalis* in the northern North Sea, where it is largely restricted to the 'Offshore zone', the southern limit being marked by the 60 m line. Low salinity is probably responsible for its absence from the southern North Sea; and in general it seems limited more by hydrographic conditions than by the relative abundance of foraminiferan food. For example, in Stephen's *Thyasira* + Foraminifera Zone, it is no more than sparsely present. Holme (1953) finds *Dentalium entalis* rare or absent near Plymouth, though it was relatively common at the Eddystone Grounds at the end of last century (Allen, 1899). It has today receded to the mouth of the Channel, in coarse deposits resulting from fairly strong scour, and Holme suspects it to be one of the list of species sensitive to recent hydrographic changes in the area.

For the living material used in this study I am greatly indebted to Mr N. A. Holme, who collected it in deeper water in the Celtic Sea on a 1953 cruise of the R.R.S. 'Discovery II'. With it were collected *Chlamys opercularis*, *Caryophyllia* and in places *Astropecten irregularis*; there were also many of the tusk-shaped serpulid tube worm *Ditrupa arietina* which as I found can impose a neat deception on the uncritical hunter for scaphopods.

Dentalium entalis survived for some months in fairly clean sand of medium grade under circulation in the laboratory, settling happily only when it was able to burrow obliquely into the substrate with about a centimetre of the narrow tip exposed. As Yonge (1937) has shown, both the inhalant and exhalant pallial currents pass through the small pallial tube that projects at that end of the shell. Apart from Yonge's paper on the ciliary currents and water circulation of the pallial cavity, little has been published in detail on the mode of life of scaphopods, though it has been known for many years, at least since Clark (1849), that they feed on Foraminifera. The purpose of this paper is to give a more detailed account, from living material, of the organs responsible for capturing and digesting food.

The concavely curved side of the shell is dorsal and in burrowing—whatever way it begins—the animal will soon twist until the dorsal side is uppermost in the obliquely buried position (Fig. 1 F). When *Dentalium* is withdrawn into its shell the mantle cavity is closed in front except for a narrowly constricted central pore; within this, the tongue-shaped foot is retracted, forming a slightly curved muscular column lying ventrally to the head and filling most of the anterior third of the mantle cavity. As burrowing begins, the foot is protruded through the mantle pore as a firmly pointed lobe, which extends until it is fully external, encircled by the widely distended mantle orifice around its base. The foot has an erectile fold of skin, narrowest in the mid-line above and below and expanding into broad rounded lobes at either side. As the tip of the foot is plunged into the ground at each burrowing stroke these side lobes are retracted, until of small size, pressed close to the foot and forced into the sand with it. The extended foot is then engorged with blood from the general haemocoele, and the lobes are strongly erected, their edges being recurved to form a wide circular flange, anchoring the animal firmly in

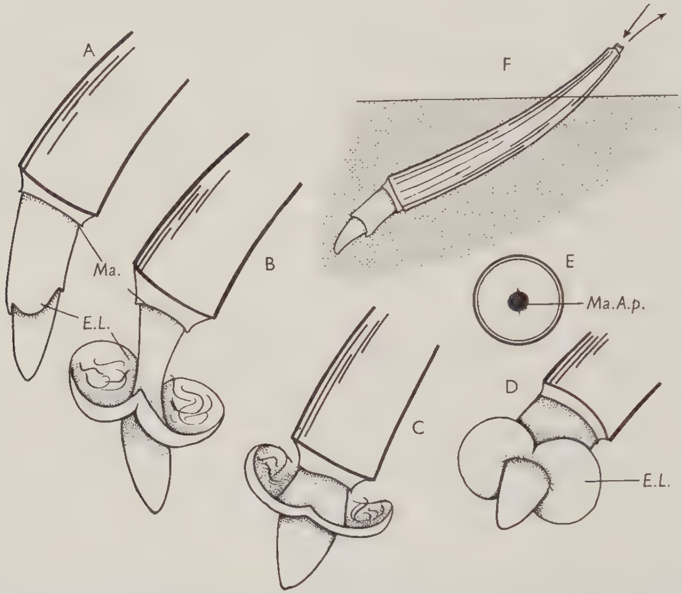


Fig. 1. *Dentalium entalis*: A, B, C, successive stages in the burrowing of the animal, showing the action of the foot and its extensible lobes. D, the extended foot viewed more anteriorly. E, the anterior end with the foot retracted showing the contracted pallial aperture. F, the shell and animal in natural posture in the sand. *E.L.*, extensible side lobes of the foot; *Ma.*, edge of the mantle with its aperture distended; *Ma.A.p.*, contracted pallial aperture.

the loose substrate. The stalk of the foot is now shortened as the shell is drawn up behind, and it is usually at the first such move that a twist is made through up to 90° as the animal orients dorsal side up. When the animal is suitably embedded the foot is partly retracted through the dilated pore of the mantle, and the animal puts out large numbers of the delicate cephalic appendages or captacula, which thrust their way in all directions through the surrounding sand.

THE CAPTACULA

These filiform prehensile tentacles arise in a dense bunch, from a single lobe (which may be partly divided) at the outer base of the proboscis on either side. Each is enlarged at its tip into a small ovoid or flattened bulb. They can elongate greatly as they are protruded and show a great deal of autonomous writhing movements when detached, but when retracted they lie in a compact cluster at either side between the proboscis and the mantle wall. They vary somewhat in number (in a typical cluster I counted about 135 on one side) and also in size; some, in the resting state, reach to the tip of the foot, others—probably recently regenerated—are short and their bulbs almost sessile. Extrusion takes place by the inflow of blood, and the captacula radiate in large numbers like the finest of strands through the substrate; rapid withdrawal is achieved by the contraction of their longitudinal muscle fibres. Though their action has never been described fully, they constitute the only food-catching organs, searching out and skilfully locating Foraminifera: there is seldom any admixture of detritus or of other living animals with the diet, and the *Dentalium* seems a strongly specialist feeder, though—as Clark (1849) has mentioned—it may sometimes vary its diet with bivalve spat or large diatoms. Once or twice I found the broken-off tip of a captaculum among the contents of the proboscis, still attached to a foraminiferan; and Dell (1957) has for a New Zealand species found Foraminifera entangled in the extruded captacula, as well as in the proboscis. *Dentalium* is nevertheless so shy in captivity that I could never once observe it in the act of taking food, and I can find no account in the literature bearing the stamp of actual observation.

For some understanding of the way the captacula work we need an account of their anatomy. From the size of these organs in relation to the bulk of a foraminiferan it seems a fair inference that a group of captacula comprising some numbers must attach together to each organism to be hauled in. Fol (1889) has given an account of the structure of the captacula with which I must in some points disagree. Each tentacle—as he showed clearly—is provided with means of extension and retraction and contains at its tip secretory glands, a ganglion and sensory receptors. Through the filament runs a central blood-filled cavity connected with the haemocoele of the head and surrounded by a layer of strong and regular longitudinal muscle fibres, usually ten in number. The central cavity has no internal epithelium as figured by Fol: the small

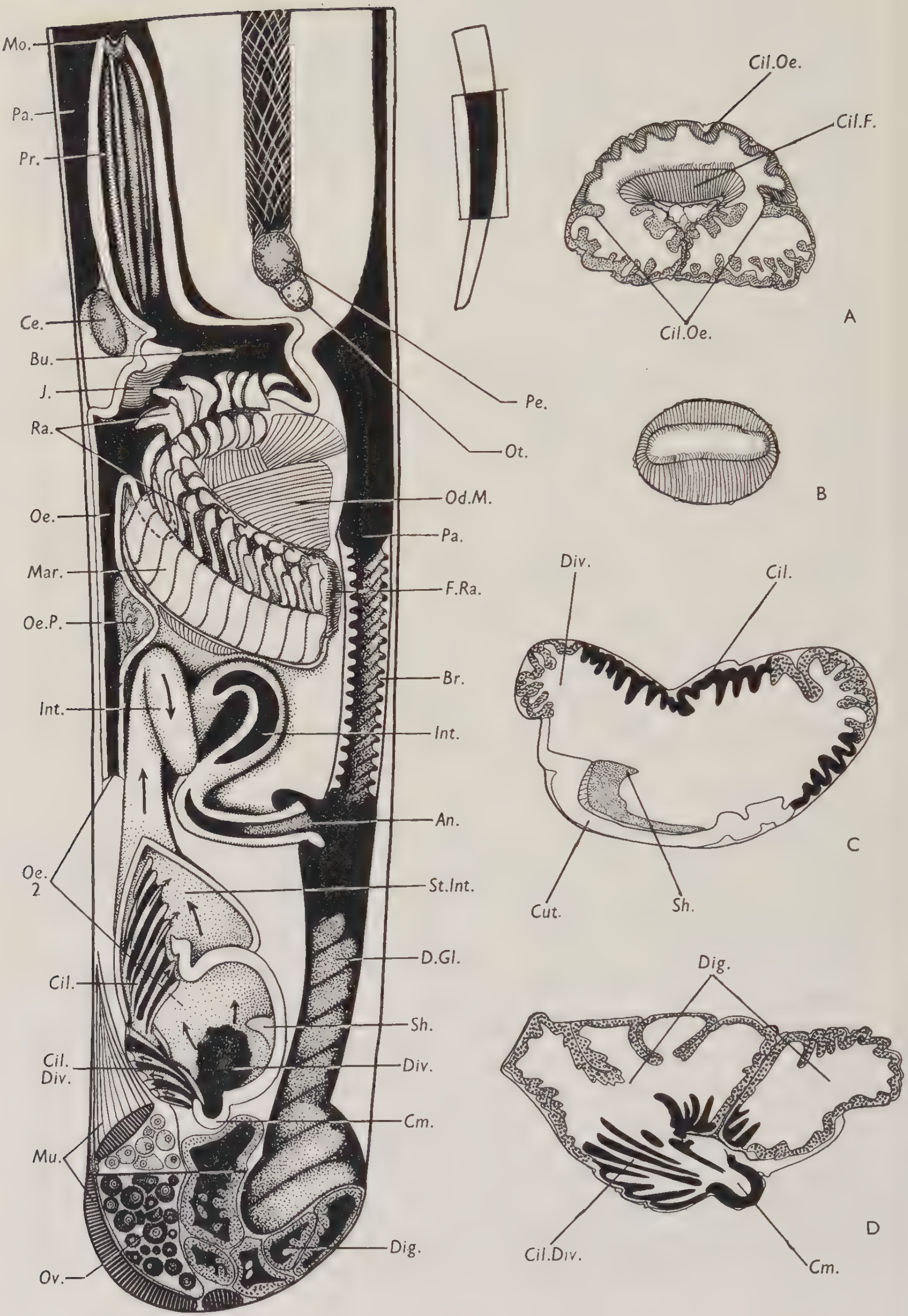

Mo.
Pa.
Pr.
Ce.
Bu.
J.
Ra.
Oe.
Mar.
Oe.P.
Int.
Oe.
2
Cil.
Cil.
Div.
Mu.
Ov.
Pe.
Ot.
Od.M.
Pa.
F.Ra.
Br.
Int.
An.
St.Int.
D.Gl.
Sh.
Div.
Cm.
Dig.
Cil.Oe.
Cil.F.
Cil.Oe.
A
B
Div.
Cil.
Cut.
Sh.
C
Dig.
Cil.Div.
Cm.
D

Fig. 2

dark-staining ovoid nuclei appearing there belong to blood amoebocytes or to a thin reticulum of connective tissue, especially concentrated near the slender nerve that runs through one side of the cavity. No circular muscles can be found; instead the filament is invested at close intervals by extremely slender connective tissue fibres. These are overlaid by a delicate squamous epidermis whose elongate nuclei run transversely to the filament. When the filament is contracted these cells stand out as regularly spaced annulae along its surface. The captaculum shows a rather close parallel to the echinoderm tube foot, now well understood from the work of Smith (1947), and forming essentially a tentacle with a hydroskeleton, longitudinal muscles and an outer circular investment of collagen. During elongation (in *Dentalium* by blood, in echinoderms by ambulacral fluid) increase in diameter is moderated by the limited elasticity of the circular elements while the longitudinal muscles are extended. When the longitudinal muscles contract to bring about retraction, the circular fibres must be sufficient to prevent distention of the wall by fluid under pressure, the attenuated cylinder having a minimal ratio of volume to restraining wall surface.

In none of the numerous sections examined could I find any trace of Fol's 'bandelette vibratile longitudinale' on one flattened side of the filament. The epidermal cells run right round the filament and it is difficult to see how such

---

Legend to Fig. 2

Fig. 2. *Dentalium entalis*. *Left*. Somewhat schematic view of the middle part of the body, displaying the left half in approximate sagittal section to illustrate the course of the alimentary canal and its relation to the surrounding structures. The dorsal side is to the left. At the posterior end, the left half of a transverse section is revealed, with the retractor muscle and ventral mantle wall continuing a little to the right of the middle line. A portion of the oesophagus is omitted, where it inclines to the right before entering the stomach. × 10. *Inset*. The shell at natural size, showing the extent of the parts illustrated. *Right*. Transverse sections of successive parts of the alimentary canal. A, the oesophagus, through the region of the oesophageal pouches; B, the oesophagus behind the pouches; C, the stomach, near the anterior limits of the digestive diverticulum; D, the posterior end of the stomach passing through the caecum and adjacent digestive gland and cutting tangentially the ciliated tracts associated with the caecum and diverticula. *An.*, anus; *Br.*, folds of the mantle wall serving as the respiratory organ; *Bu.*, buccal cavity; *Ce.*, cerebral ganglion; *Cil.*, ciliated fold of the roof of the stomach leading to the intestine; *Cil.Div.*, ciliated folds associated with the caecum and digestive diverticula; *Cil.F.*, ciliated fold of the glandular region of the oesophagus; *Cil.Oe.*, ciliated lining of the oesophagus; *Cm.*, caecum of the stomach; *Cut.*, cuticulated epithelium of the stomach; *D.Gl.*, digestive gland tubules spreading round the side of the mantle cavity; *Dig.*, digestive gland in section; *Div.*, digestive diverticulum leading from stomach; *F.Ra.*, formative cells of the radula at the bottom of the radular sac; *Gl.Oe.*, limits of the glandular region of the oesophagus; *Int.*, intestine; *J.*, jaw; *Mar.*, marginal teeth of left side within the radular caecum; *Mo.*, mouth; *Mu.*, retractor muscles attached posteriorly to the shell; *Od.M.*, muscles of the odontophore; *Oe.*, oesophagus; *Oe.P.*, oesophageal pouch; *Oe.* 2, extent of interrupted section of the oesophagus; *Ot.*, otocyst; *Ov.*, ovary; *Pa.*, pallial cavity; *Pe.*, pedal ganglion; *Pr.*, proboscis, *Ra.*, radula (lateral teeth of left side and median teeth); *Sh.*, vestige of gastric shield; *St.Int.*, anterior part of stomach, tapering forward to intestine.

a ciliated tract could be maintained with continual alterations of length, or what function it could perform.

The tip of the captaculum (Fig. 3A)—unlike the filament—is ciliated all over, but especially densely in a subterminal alveolus or shallow depression lying on one side, where the cilia are twice as long as elsewhere. The interior of the terminal bulb is filled mostly by a close-packed mass of ovoid dark-staining epidermal nuclei, surrounding near the centre a small ganglion presumably connected with the captacular nerve. Associated with this ganglion Fol clearly figures rod-like end-organs between the columnar cells lining the alveolus. At the base of the terminal bulb where the longitudinal muscles taper off, lies a nest of plump-bodied epidermal glands, with basal nuclei and their contents non-staining save for clusters of granules which become red in azan and black in iron haematoxylin. Some of these glands were seen in sections to be discharging at the surface of the epidermis.

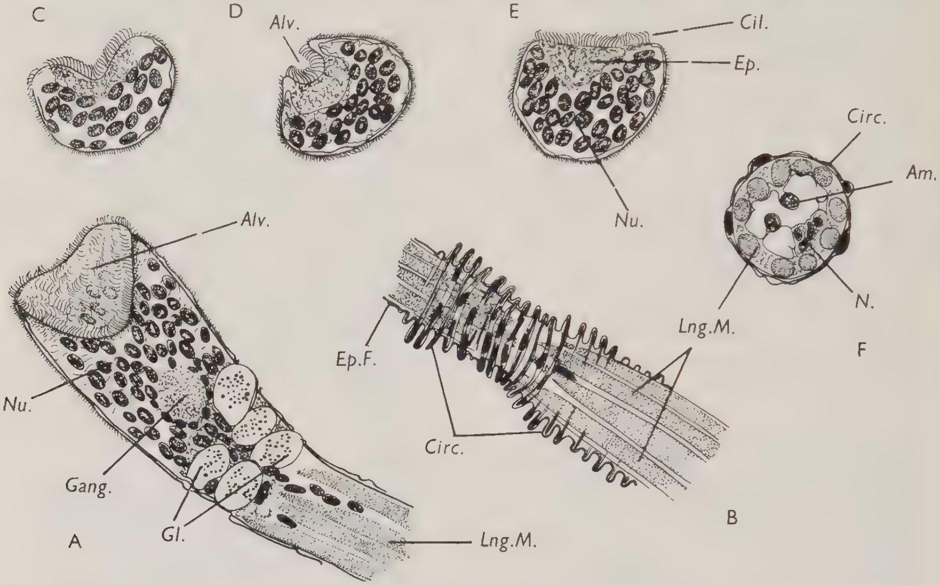


Fig. 3. *Dentalium entalis*. Histology of the captacula, from sections cut at 5 μ and stained in iron haematoxylin or in Heidenhain's azan. A, the tip of a captaculum in tangential section, showing part only of the ciliated alveolus, the ganglion and the nest of gland cells; B, tangential longitudinal section of part of a filament showing the arrangement of longitudinal muscles and circular connective tissue; C, D, E, transverse sections of the terminal bulb with different conditions of the ciliated alveolus, from contraction to the flatly expanded state; F, transverse section of a filament. *Am.*, amoebocyte in lumen; *Alv.*, alveolus; *Cil.*, cilia; *Circ.*, circular connective tissue; *Ep.*, epithelium of the alveolus; *Ep.F.*, epithelium of the filament; *Gang.*, ganglion; *Gl.*, glands; *Lng.M.*, longitudinal muscle; *N.*, nerve; *Nu.*, nuclei of epithelial and sensory cells of terminal bulb.

By what means can the captacula take hold of a foraminiferan test? It is unlikely from their situation that the gland cells help in prehension; it is tentatively suggested these may produce a toxin disabling living Foraminifera, or even a repugnatorial secretion protecting the animal's own widely deployed and otherwise vulnerable captacula. There are no obvious prehensile structures, in the form of organised suckers. A viscous secretion seems unlikely, from the histological structure of the bulbs, and although the filament can sometimes coil, it is impossible to visualize it as an entwining tentacle. As shown in Fig. 3, the subterminal depression of the bulb may be contracted to a deep cup, or be opened out shallowly, or perfectly flat in its full state of expansion. These changes of shape must be secured by a fine intrinsic musculature, which was, however, quite obscured by the crowded nuclei in the preparations illustrated. Closely applied to the foraminiferan test, when flattened out, the depression could provide a temporary but quite effective suction cap. Several captacula acting in concert could obviously exert a significant pull quite adequate to bring the foraminiferan to the mantle cavity and thence to the mouth. By its coat of cilia the tip of the captaculum might be moved over the surface of the test, exploring it for position before taking up its attachment. A parallel to such a mode of attachment is already known, in the tentacles of the terebellid polychaete worms, as carefully described by Dales (1955). These though much larger have a somewhat comparable structure, each forming a long, very extensible fluid-filled appendage traversed by longitudinal muscles. Instead of a terminal depression they have a ciliated groove all the way along; this can be applied locally to the substrate and creep along by its cilia, and, when the groove is locally flattened out, the tentacle can take a firm hold of the ground at any point along its length.

ALIMENTARY CANAL

The gut begins with a flattened muscular-walled proboscis, contractile but not inversible, projecting from the head dorsally to the foot and lying in front of the buccal mass between the bunches of captacula. The mouth is a horizontal slit running across the anterior end of the proboscis, and its sides are drawn out to give it a wide crescentic form. It has thickened, crenulated lips, and when the captacula are withdrawn into the mantle cavity, the tips of the longest come to lie about level with the mouth. From the position of some captacula in fixed material, it seems they may be drawn through or between the parted lips, as their attached load is ingested. After feeding, the muscular wall of the proboscis is then stretched quite thin, and its cavity is crammed with the tests of living Foraminifera, which bulge out all over its surface like small close-set pustules or sometimes angular projections. When full, it was found to contain a dozen or so large tests (up to $350\,\mu$), three or four times as many small ones, and almost nothing more. Such fine power of selectivity is a testimony to the searching efficiency of the captacula. An occasional diatom frustule may be picked up, and at times empty foraminiferan tests, but the great bulk of the meal is of living Foraminifera, all perfectly intact in the proboscis, trituration taking place further back in the gut. A specimen from the Celtic Sea, with the proboscis full, had for example collected the following haul:

Elphidium sp. (=*Polystomella*), the largest and commonest species, about six tests up to 350 μ, and many smaller ones.

Quinqueloculina sp., a miliolid, two large tests (200 μ) and a number of small.

Discorbis (?) sp., a trochoid rotaliid.

Globigerina test, apparently empty when picked up and not recorded living in this area.

A Millport preserved specimen examined for comparison had the proboscis filled with a single species of *Bulimina*.

I am indebted to Dr R. H. Hedley for identifying the Foraminifera as accurately as possible after decalcification resulting from fixation.

Behind the proboscis lies a buccal mass, of about the same length but much deeper and more muscular. The proboscis is very contractile and may empty its contents by peristalsis, while removal of Foraminifera may be helped by the radula which is so situated that its lateral teeth when protracted could haul whole frustules, perhaps several at a time into the buccal mass. Fig. 4 shows the relative size and proportions of the radula, typical foraminiferan tests, and captacular bulb. The radula is relatively immense by comparison with the rest of the gut, and is proportionately larger than in any other mollusc I have seen. It has approximately 18 rows of teeth, each bearing 5 teeth. The central tooth is a crescentic transverse plate with no sharp cusps. It is flanked at each side by a strongly falcate lateral tooth, and outside that by a smooth marginal tooth, little more than a broad plate of chitin paving the side of the odontophore. The radula has the usual morphological relations: it lies in a narrow groove at the centre of the odontophore, and is spread out for protection, and afterwards retracted, by muscles attached to an odontophoral skeleton of two 'cartilages'. The laterals are the main functional teeth, and their distal expansions are wide and hood-shaped behind the sharp tip. A foraminiferan test grasped by them and lifted up towards the roof of the buccal mass might then be engaged between the strong bar of the central tooth and the chitinous jaw. This is a median, bow-shaped plate, running across the front wall of the buccal mass. As well as transferring Foraminifera from the proboscis to the oesophagus, the radula may thus also have acquired the task of fracturing and triturating them. This would accord with its uniquely large size in *Dentalium*, the lack of other hard triturating surfaces farther back, and absence, in my material, of any intact frustules in later parts of the gut. Clark, however, mentions finding them in the stomach, and the very contractile stomach wall must play some part as a gizzard when distended by the food mass.

Of the rest of the gut the chief features for remark are the absence of mucus

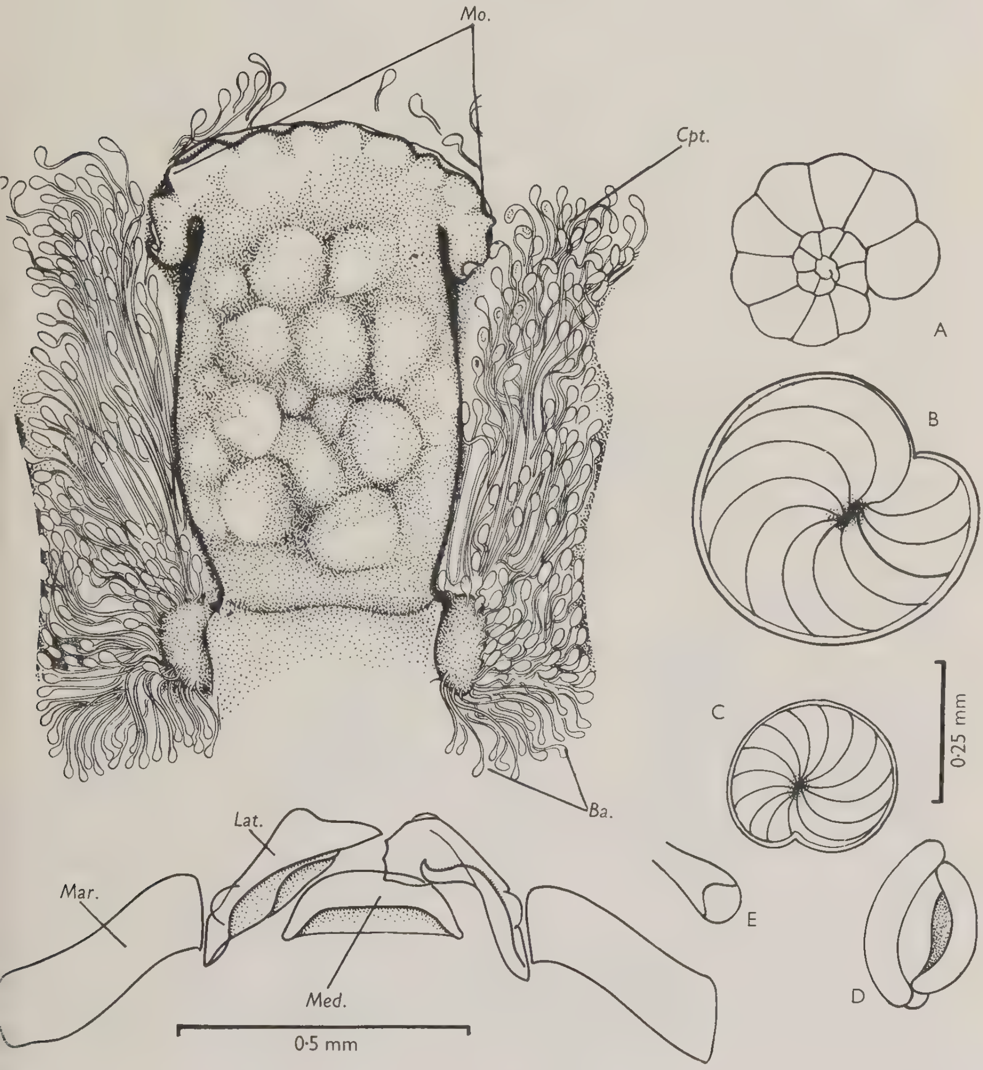


Fig. 4. *Dentalium entalis. Upper left.* The proboscis and the bunches of retracted captacula viewed from above, after the removal of the dorsal mantle wall. Ingested foraminiferans bulge through the wall of the proboscis. *Lower left.* A single row of teeth from the radula. *Right.* To the same scale as the radula are shown outlines of representative foraminiferans found in the proboscis: A, *Discorbis* sp.; B, C, *Elphidium* sp. of different sizes; D, *Quinqueloculina*. The tip of a captaculum, E, is also represented to the same scale. *Ba.*, short captacula at the base of the cluster; *Cpt.*, captacula; *Lat.*, lateral tooth; *Mar.*, marginal tooth; *Med.*, central tooth; *Mo.*, extent of mouth.

secretion (except for a few cells on the folds leaving the buccal mass), the loss of salivary glands, the lack of digestive amoebocytes and the occurrence of extracellular digestion. As the oesophagus runs back, a large flat-topped ciliated fold, forming its floor, becomes thinly constricted at its attachment. The spaces so developing at its sides enlarge into a pair of oesophageal pouches, the apparent homologues of those of Polyplacophora and Archaeogastropoda. Their lining epithelium is as shown in Fig. 5 B, sparsely ciliated, with a striated border and with tiny spherical inclusions, black in iron haematoxylin, yellow in azan. Though fixed cells as seen in sections tend to 'bubble' and carry clusters of spherules into the lumen, I am not sure that they are an

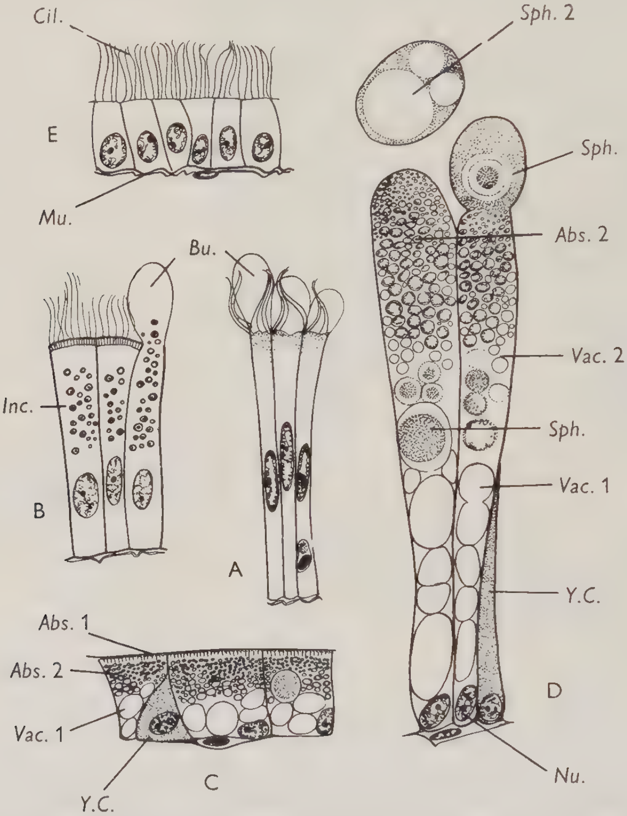


Fig. 5. *Dentalium entalis*. Histology of the alimentary canal. A, ciliated cells of the oesophagus; B, glandular cells of oesophageal pouch; C, cells of digestive gland at early absorbing stage; D, digestive cells of maximum size at late stage, producing 'secretory' spheres; E, cells of intestine. *Abs.* 1, finely striated flat absorbing surface of the digestive cell; *Abs.* 2, absorbed material not yet enclosed in cell vacuoles; *Bu.*, 'bubbling' cytoplasm of oesophageal cell; *Cil.*, cilia; *Inc.*, granular inclusions in oesophageal cell; *Mu.*, muscle fibre; *Nu.*, nucleus; *Sph.* 1, sphere rounding off at tip of mature digestive cell; *Sph.* 2, sphere cut off from cell; *Vac.* 1, colourless vacuoles towards base of cell; *Vac.* 2, digestive vacuoles with absorbed contents; *Y.C.*, young cell.

important source of secretion in life—similar types of inclusions may indeed be seen in the non-glandular cells beneath the cuticle of the stomach. Behind these pouches the oesophagus narrows to run as a ciliated tube to the right side of the stomach, close to where the intestine emerges towards the left.

The stomach, though thin-walled, has a muscular tunic and performs vigorous contractions in life. It probably serves chiefly as a gizzard into which open posteriorly at either side the very wide, almost symmetrical, apertures of the paired digestive diverticula. The stomach is a globular or pyriform sac, with little more than half the volume of the buccal mass, and tapers towards the oesophagus and intestine in front. Its structure, viewed in Fig. 2, shows disappointingly little of the primitive architecture of the molluscan stomach, though there are two features that would pass unnoticed but for an awareness of the past history of this organ. A small conical papilla, ciliated inside, and projecting behind the digestive diverticula is evidently a relic of the posterior gastric caecum. Immediately to the right and left of it begin the apertures of the respective digestive diverticula; and from the caecum as a centre point a series of ciliated ridges radiate over the posterior end of the stomach. Some of these extend to the thresholds of the diverticula, while most run up to the dorsal side of the stomach, slanting towards the intestine and terminating at the summit of a flat triangular fold (Fig. 2C, D). Their ciliation is simple, producing a set of currents leading along the grooves towards the intestine. These are the only ciliary movements within the stomach: cuticle covers the floor and such part of the side-walls as is not perforated by the two diverticula. The floor is raised at one point into a small spur of cuticle, the second relic of the primitive stomach, which represents a vestige of the gastric shield.

No trace of style-sac ciliation survives, and material from the stomach is periodically squeezed into the intestine by peristalsis, in a loosely compacted rope formed of the remains of Foraminifera, held together by the viscous outflow from the digestive gland. The intestine describes a double loop in the space between the stomach and buccal mass, the rectum then descending to open in the ventral mid-line into the pallial cavity immediately behind the region of the respiratory folds. The faeces are not bulky and are not compacted into separate firm pellets. The intestinal lining cells (Fig. 5E) are shortly columnar with sparse longish cilia; and I could find no secretory cells.

DIGESTIVE GLAND

The digestive gland clearly secretes enzymes for extracellular digestion, as can be judged by the bulky nature of the food, its complete breakdown in the stomach, and the lack of any other enzyme sources with the very doubtful exception of the small oesophageal pouches. The gland is also the site of absorption. It is built up of tubules closely adpressed with little or no intervening connective tissue. Each common diverticulum opens straight out of the stomach, to break into a row of thick, finger-shaped tubules on either side. The right and left lobes of the digestive gland so formed unite in a median dorsal bridge over the mantle cavity and beneath the gonad. In either lobe, the series of tubules becomes smaller towards the front and the back of the lobe, and spreads round the mantle cavity, except for a median ventral strip which is thin and transparent.

The digestive cells absorb fluid and fine suspended material carried into the tubules by muscular contractions in the stomach. They show in local regions all stages between the two extremes of shape and size in Fig. 5C–D. The first stage is low and squarish, just beginning to absorb, with large clear basal vacuoles and finely granular vacuoles in the distal cytoplasm, whose contents stain blue or grey in azan, identically with the contents in the lumen. Later cells become greatly attenuated, widening and bulging at the loaded tips. The vacuoles in the basal half are still mostly clear, but distally they are loaded with ingested material. The tips are now rounding into compact spheres that will be nipped off and returned to the stomach, entering in large numbers and easily detectable in the faeces. They rarely contain a nucleus and such 'secretion' is merocrine rather than holocrine. This suggests that the cells may again become absorptive, but the digestive gland is also provided with young 'replacing cells' that have not yet begun to absorb. These are broad-based (5 C) or narrowly trangular (5 D) with rather large nuclei and uniformly dark-staining cytoplasm. As well as bluish vacuoles (azan) the fragmenting tips of the mature cells occasionally contain a vacuole filled by a single large greenish yellow droplet, probably excretory. The contents of all the extruded vacuoles must represent the residual waste returned to the stomach after digestion and assimilation of food. It must be further assumed that the abstricted spheres also provide, either in their vacuoles or in the residual cytoplasm, such enzymes as are necessary for extracellular digestion. The cells thus pass through a life-history widespread in mollusc digestive cells (see Morton, 1956, and Owen, 1955, for lamellibranchs, where there is usually no secretion, and Bidder, 1950, for cephalopods). In *Dentalium* the cycle includes absorption, assimilation, extrusion of indigestible and excretory residues, and the provision of extracellular enzymes in advance of the next meal.

The Scaphopoda must have early left the main road of advance, being known as a separate group since Silurian times. They retain a primitive simplicity of the renal organs, absence of separate genital ducts and external fertilization, together with a much more complete gut than in lamellibranchs. Even more than the lamellibranchs they have become deeply committed to burrowing life, and—as in the bivalves—the mouth is raised out of direct contact with the substratum. Feeding, which in most modern lamellibranchs has devolved on elaborate gills and in Nuculacea upon palp proboscides, depends in Scaphopoda upon the unique head appendages, the captacula, and on the immensely developed radula, which in the bivalves is entirely lost. The lamellibranch gills and mantle cavity equipped their possessors for lavish and long-continued radiation; the scaphopod captacula, by contrast, at once restricted the possible range of diet and variety of habits—in evolutionary terms they are the badge of failure. *Dentalium* has no gills; the annular pallial folds are adequate for its respiratory needs, and their powerful cilia sufficient to maintain the inhalant and exhalant posterior currents. With a simplified respiratory system, the circulation is in turn rudimentary; there is no heart, but a simple contractile area of the haemocoele wall near the anus. The rhythmic expansion and contraction of the foot and its periodic charging with blood must have far more influence upon blood circulation than could the relatively feeble action of a heart.

SUMMARY

The scaphopod *Dentalium entalis*, studied with specimens from the Celtic Sea, is a specialized burrower in medium to coarse sands and its chief or only diet is of Foraminifera. Burrowing is performed by a muscular, very extensible, tongue-like foot, protruded through the anterior pore of the mantle cavity, and anchored in the sand at each distensible burrowing thrust by erectile side lobes. The foraminiferans are captured by very numerous extensible head tentacles or captacula. These radiate in the substrate and terminate in an expanded bulb, with gland cells, receptors and a ciliated alveolus which is able to be flattened out to provide—it is suggested—an attachment organ. Collected Foraminifera are hauled from the cavity of the proboscis into the buccal cavity by a large, strong radula, and pass along the oesophagus to the stomach. This is simplified in structure with a thin but muscular wall which serves the function of a gizzard. There is a vestigial caecum and gastric shield. The digestive diverticula open very widely from the stomach. Digestion appears to be wholly extracellular, and the digestive tubules are compared in structure with those of other Mollusca. The intestine is a simply coiled tube and the faeces are small and uncompacted.

REFERENCES

ALLEN, E. J., 1899. On the fauna and bottom deposits near the thirty fathom line from the Eddystone to Start Point. *J. mar. biol. Ass. U.K.*, Vol. 5, pp. 365–542.

BIDDER, ANNA M., 1950. The digestive mechanism of the European squids. *Quart. J. micr. Sci.*, Vol. 91, pp. 1–43.

CLARK, W., 1849. On the animal of *Dentalium tarentinum*. *Ann. Mag. nat. Hist.*, Ser. 2, Vol. 4, pp. 321–30.

DALES, R. P., 1955. Feeding and digestion in terebellid polychaetes. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 55–79.

DANOIS, E LE., 1948. *Les Profondeurs de la Mer.* Paris: Payot.

DELL, R. K., 1957. A revision of the recent scaphopod Mollusca of New Zealand. *Proc. roy. Soc. N.Z.*, Vol. 84, pp. 561–76.

Fol, H., 1889. Sur l'anatomie microscopique du Dentale. *Arch. Zool. exp. gén.*, T. 7, pp. 91–148.

Holme, N. A., 1953. The biomass of the bottom fauna in the English Channel off Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 1–49.

Morton, J. E., 1956. The tidal rhythm and action of the digestive system in *Lasaea rubra. J. mar. biol. Ass. U.K.*, Vol. 35, pp. 563–86.

Owen, G., 1955. Observations on the stomach and digestive diverticula of the Lamellibranchia. I. The Anisomyaria and Eulamellibranchia. *Quart. J. micr. Sci.*, Vol. 96, 517–37.

Smith, J. E., 1947. The activities of the tube feet of *Asterias rubens*. *Quart. J. micr. Sci.*, Vol. 88, pp. 1–14.

Stephen, A. C., 1933. Studies on the Scottish Marine Fauna: The natural faunistic divisions of the North Sea as shown by the quantitative distribution of the molluscs. *Trans. roy. Soc. Edinb.*, Vol. 57, pp. 601–16.

Yonge, C. M., 1937. The circulation of water in the mantle cavity of *Dentalium*. *Proc. malacol. Soc. Lond.*, Vol. 22, pp. 333–8.

J. mar. biol. Ass. U.K. (1959) 38, 239–248
Printed in Great Britain

FEEDING IN NUDIBRANCH LARVAE

By T. E. Thompson

Marine Biological Station, Port Erin, Isle of Man

(Text-figs. 1–7)

No account exists of the mechanism of food collection of nudibranch larvae, and, in fact, it has been considered unlikely that feeding does take place in these forms. Thorson (1946, p. 275) states 'Nudibranch larvae will normally take no—or only little—food from the plankton'.

Observations by the present author on larvae of the dorid nudibranch *Adalaria proxima* (Alder & Hancock) showed clearly that, although further development with metamorphosis will occur in this species regardless of whether food is provided for the larvae, a feeding mechanism is present and functions throughout pelagic life (Thompson, 1958). Moreover, sections through the larval digestive gland revealed the presence of food vacuoles, proving the ability to assimilate planktonic micro-organisms. These observations provided a stimulus to further work in order to ascertain whether the feeding mechanism described for *Adalaria* was present in other nudibranchs which possess a free-swimming larval phase.

Accounts of the anatomy of nudibranch veligers are few. Pelseneer (1911) figured many species, but made little attempt to ascribe functions to the various organs. Rasmussen (1944, 1951) gives more detailed figures of the larvae of a few nudibranchs, but again goes into little functional detail, and fails to orientate the veliger correctly (he repeatedly states that it is the *right* midgut diverticulum which is the larger whereas his figures leave no doubt that this is incorrect).

MATERIAL AND METHODS

Vestergaard & Thorson (1938) and Thorson (1946) divide nudibranch larvae into three types, according to the shape of the larval shell. In the present investigation, only species whose larvae belong to Thorson's shell-types B and C have been treated. There can be little doubt that the type A does not constitute a natural division, but represents a type B shell at an early stage of development (own unpublished observations on *Tritonia hombergii* Cuvier). The differences in structure between larvae of shell-types B and C, however, are far more striking and significant. Fig. 1 shows these two nudibranch larval shell types. The type C shell is confined to the families Dendronotidae, Eubranchidae, Tergipedidae and Fionidae; six families, Calmidae, Proctonotidae, Arminidae, Scyllaeidae, Hancockiidae and Lomanotidae have yet to be investigated; members of all the other British families which have been studied (see below, and Thorson, 1946, p. 269) possess larval shells of type B. (For the reason stated above, the family Tritoniidae is here considered to consist of forms with shells of type B.) In only one instance does it appear that a family contains species with both type B and type C shells: *Coryphella rufibranchialis* is said by Thorson (1946) to have a larval shell of type C, whereas *C. lineata* (see below) has a shell of type B. It seems probable that subsequent work will show one or the other of these observations to be incorrect.

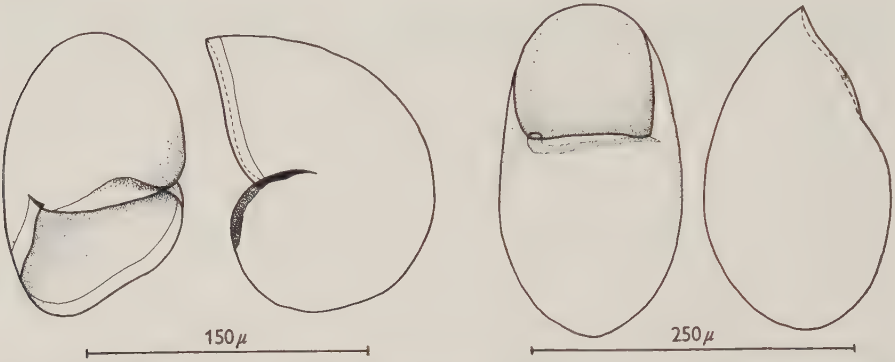


Fig. 1. Nudibranch larval shells of Thorson's types B and C: left, postero-ventral and lateral views of *Jorunna tomentosa* (type B); right, ventral and lateral views of the larval shell of *Trinchesia aurantia* (type C).

As a representative of the nudibranch larvae with shells of type B, *Archidoris pseudoargus* (Rapp) (Glossodorididae) has been investigated in detail, while less detailed observations were made on the following: *Jorunna tomentosa* (Cuvier) (Glossodorididae), *Onchidoris fusca* (Müller) (Limaciidae), *O. muricata* (Müller) (Limaciidae), *Polycera quadrilineata* (Müller) (Limaciidae), *Goniodoris nodosa* (Montagu) (Limaciidae), *Acanthodoris pilosa* (Müller) (Limaciidae), *Hero formosa* (Loven) (Heröidae), *Doto coronata* (Gmelin) (Dotöidae), *Facelina auriculata* (Müller) (Facelinidae), *Coryphella lineata* (Lovén) (Coryphellidae), *Limapontia depressa* Alder & Hancock (Limapontiidae), *Alderia modesta* (Lovén) (Stiligeridae).

As representatives of the shell-type C, observations were made on larvae of *Trinchesia aurantia* (Alder & Hancock) (Tergipedidae) and *Eubranchus exiguus* (Alder & Hancock) (Eubranchidae).

The nomenclature and classification employed are those advocated by Winckworth (1932, 1951).

All larvae were obtained from egg masses laid and reared in the laboratory. Feeding currents were observed in suspensions of fine carmine particles or of the diatom *Phaeodactylum tricornutum* Bohlin (from a culture kindly provided by Dr J. M. Kain). Larvae were fixed in hot Perényi's fluid, cleared with amyl acetate (Barron, 1934) and sectioned at 5–10 μ. Stains employed included Mayer's haemalum, Heidenhain's iron haematoxylin and, as counterstains, eosin and alcian blue 8 GS (Steedman, 1950).

THE LARVA OF *ARCHIDORIS PSEUDOARGUS*

The figures display most of the results of this investigation. Figs. 2 and 3 show the extended veliger larvae of the species, Fig. 4 the cephalopedal ciliary apparatus and Figs. 5 and 6 the alimentary canal. Only those features which are not immediately apparent from these figures will be described.

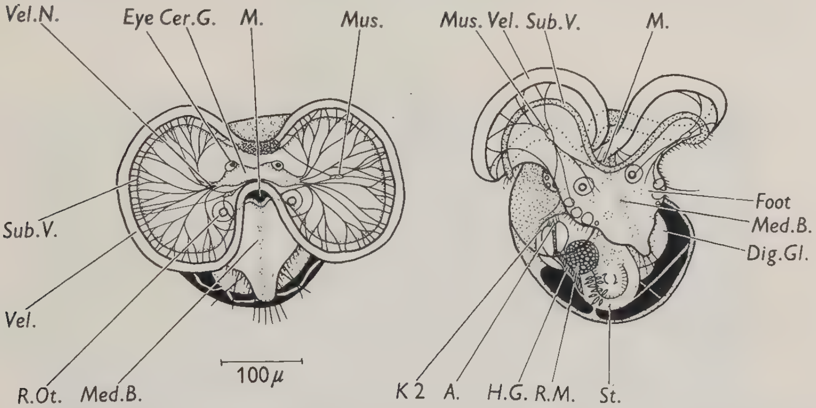


Fig. 2. Eyed larva of *Archidoris pseudoargus*, 3 days after hatching: left, viewed from the anterior; right, right latero-ventral aspect (velar locomotor cilia not illustrated). *A.*, anus; *Cer. G.*, cerebral ganglion; *Dig. Gl.*, digestive gland; *H.G.*, hindgut; *K*2, larval kidney; *M.*, mouth; *Med. B.*, median pedal band of strong cilia producing the main rejection current; *Mus.*, muscle cell; *R.M.*, right midgut diverticulum; *R.Ot.*, right otocyst; *St.*, stomach; *Sub. V.*, subvelar ridge or subvelum; *Vel.*, velum; *Vel. N.*, nerve fibres from the cerebral ganglia to the velar cells.

The cephalopedal ciliary apparatus (Fig. 4)

The long velar cilia serve a dual purpose in both imparting a forward motion to the larva and bringing a constantly renewed supply of sea water within the influence of the feeding apparatus. Particles brought within the influence of the cilia arranged along the subvelar ridges (Figs. 2–4, *Sub. V.*) are rapidly conducted towards the mouth. The borders of the mouth (Figs. 2, 4, *M.*) are strongly ciliated and particles of sufficiently small size (less than *ca.* 15 μ in diameter) are impelled into the foregut.

The long velar cilia can be stopped and started, being under the control of the nervous system (Carter, 1926). All the other cilia of the larval body beat continuously, and a feeble feeding current is manifest even when the larva sinks passively with the velum inactive.

The external surface of the foot is ciliated, these cilia being particularly strongly developed along a narrow zone leading from the ventral border of the mouth to the papillate tip of the foot (Figs. 2, 4, *Med.B.*). This narrow band forms the main rejection current; particles which are too large to enter the mouth appear to be tipped over its ventral border and are then removed rapidly by this strong stream.

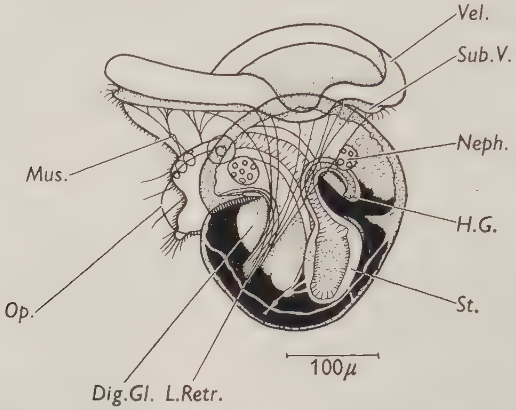


Fig. 3. Newly hatched larva of *Archidoris pseudoargus* from left latero-dorsal aspect (velar locomotor cilia not illustrated). *Dig. Gl.*, digestive gland; *H.G.*, hindgut; *L. Retr.*, larval retractor muscle; *Mus.*, muscle cell; *Neph.*, nephrocyst; *Op.*, operculum; *St.*, stomach; *Sub. V.*, subvelar ridge; *Vel.*, velum.

The foregut (Fig. 5, *F.G.*)

The foregut is strongly ciliated internally, these cilia creating a current which conveys particles from the mouth into the stomach.

The stomach and its diverticula (Fig. 5)

The stomach is internally ciliated throughout. Short, fast-beating cilia are arranged on a raised band (Figs. 5, 6, *Cil.B.*) which causes a rapid rotation of particles in the lumen. The direction of rotation was the same in all the individuals observed. Ventrally, close to the openings of the foregut and the midgut diverticula into the stomach (Fig. 6A, *F.G.op.*, *R.M.op.*, *Dig.Gl.op.*), the cilia are longer and more slow moving. They induce an apparently aimless particle agitation, which may on occasion be seen to become more co-ordinated and take the form of a rotatory movement in the ventral stomach. A small zone of hyaline, rod-like bodies is present in the ventral stomach wall on the right side (Fig. 6B, *Hy.Rods*).

The midgut diverticula are a pair, lying symmetrically against the antero-lateral walls of the stomach. Both possess lumina; this is more marked in the case of the left diverticulum which is by far the larger. The right diverticulum (Fig. 2, *R.M.*) consists of a few large yolk-laden cells at hatching; these cells decrease in size during later larval life as, presumably, the yolk is utilized. This right diverticulum, as far as could be ascertained, plays no part in digestion.

The left midgut diverticulum or digestive gland (Figs. 2, 3 and 5, *Dig.Gl.*) is pale green-brown in life, and has a wall of single-cell thickness. It has a crescentic shape, following the line of the whorl of the shell. The cells composing its wall are ciliated and are of two kinds; the majority are cubical digestive cells but there are a few larger cells (mainly lying close to the opening of the organ into the stomach) which have yolk-laden cytoplasm. The nuclei are peripherally placed within all the cells of the digestive gland. Sections through larvae which have been allowed access to *Phaeodactylum* show large numbers of ingested diatoms enclosed in cytoplasmic food vacuoles

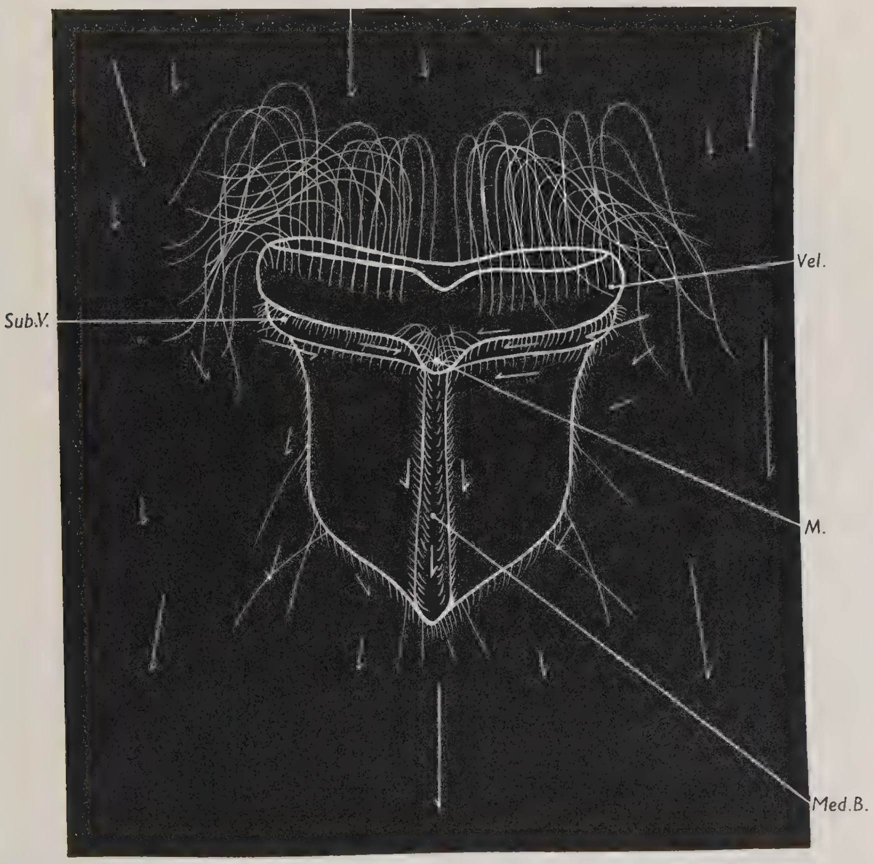


Fig. 4. Cephalopedal ciliary apparatus of larval *Archidoris pseudoargus*: semi-diagrammatic view from ventral aspect. The arrows indicate the direction in which particles are impelled. *M.*, mouth; *Med. B.*, median pedal band; *Sub. V.*, subvelar ridge; *Vel.*, velum.

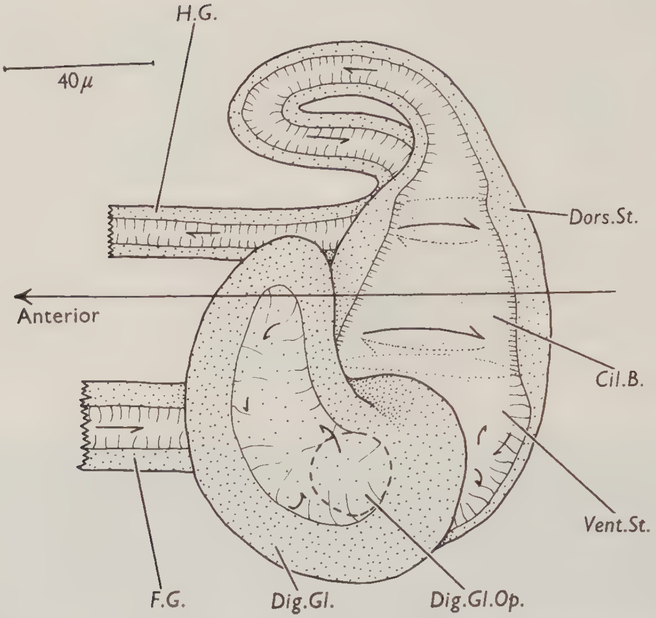


Fig. 5. The stomach and associated organs in larval *Archidoris pseudoargus*: semi-diagrammatic view from left lateral aspect. The arrows indicate the direction in which particles are impelled. In the region of the raised band of stomach cilia, the solid arrows show the direction of movement of particles in the higher focal levels whereas the dotted arrows show the movement in the lower levels. *Cil. B.*, raised band of cilia; *Dig. Gl.*, digestive gland; *Dig. Gl. op.*, opening of digestive gland into stomach; *Dors. St.*, dorsal region of stomach; *F.G.*, foregut; *H.G.*, hindgut; *Vent. St.*, ventral region of stomach.

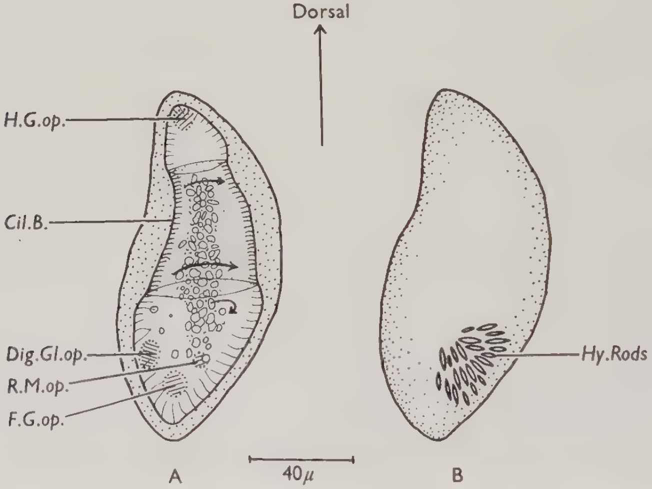


Fig. 6. The stomach in larval *Archidoris pseudoargus*: semi-diagrammatic view from the posterior aspect. A, in optical section; B, surface view only. *Cil.B.*, raised band of cilia; *Dig. Gl. op.*, opening of digestive gland into stomach; *F.G. op.*, opening of foregut into stomach; *H.G. op.*, opening of hindgut into stomach; *Hy. Rods*, zone of hyaline rod-like bodies; *R.M. op.*, opening of right midgut diverticulum into stomach.

in the digestive cells. The food vacuoles are nearly always situated close to the nuclei. Particles of carmine powder were not taken up by the digestive gland cells, as far as could be ascertained.

The hindgut (Figs. 2, 5, *H.G.*).

This is an internally ciliated tube, leaving the midgut dorsally and passing to the anus, situated in the mantle fold, latero-ventrally on the right side. Metachronal waves passing towards the stomach are always clearly evident. The anal opening is immediately ventral to the opening of the larval kidney (Fig. 2, *K*2). A pair of colourless vesicles of unknown function lie embedded in the mantle fold close to the anus (Fig. 2, *A.*). The cilia of the mantle fold and a zone of especially strong cilia on the right side of the foot (see Fig. 2) disperse the faeces as they emerge from the anus. The faeces are not cemented together into strings or pellets as they are in post-larval stages.

Course of particles entering the mouth

Particles on entering the mouth are rapidly transported by the foregut cilia into the stomach. In the stomach the raised ciliary band brings about a rapid rotation, a loose rod of particles soon being formed. The ventral extremity of this rod always is close to the patch of hyaline rod-shaped bodies in the stomach wall (see Fig. 6); it seems likely that this patch is the site of some enzymatic activity. Particles apparently haphazardly leave the rod of food particles and are impelled by the more sluggish cilia of the ventral stomach into the digestive gland. When in the lumen of the digestive gland, a proportion of them are ingested.

Millar (1955), working on larvae of *Ostrea edulis*, states that 'it is a matter of chance whether material drawn off into the midgut and thence passed to the rectum has been in the stomach for a short or a long time'; although this may be true in the larval oyster, and although no definite proof to the contrary can be provided by the present investigation, the impression was gained that a definite pattern of treatment for each new incoming particle was present, and that no particle of food value was passed directly into the hindgut unless a superabundance of such particles was present.

Passage of particles from the stomach into the digestive gland is brought about by ciliary means; nevertheless, contraction of muscle fibres in the membranes enclosing the visceral organs (Thompson, 1958) can bring about partial contraction of the organs and probably aids this interchange. Millar (1955) described a similar phenomenon in *Ostrea.*

OTHER SPECIES

In all the other nudibranch larvae examined, with the partial exceptions of *Trinchesia aurantia* and *Eubranchus exiguus*, the feeding mechanism is as described for *Archidoris pseudoargus*. Minor specific differences occur, such as the black pigmented mouth of the larva of *Limapontia depressa* or the hyaline velar granules of *Hero formosa*, but the functions of the various organs and even their relative state of development is closely similar in all. Even the direction of rotation of particles within the stomach was in all cases the same as that shown for *Archidoris* in Fig. 5. (Yonge, 1926, found that the direction of rotation of particles within the stomach of larval *Ostrea* varied from one individual to another.)

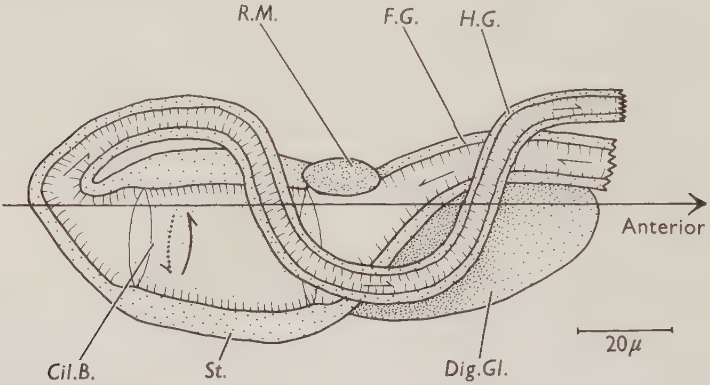


Fig. 7. The stomach and associated organs in larval *Trinchesia aurantia* from right lateral aspect. The arrows indicate the direction in which particles are impelled. In the region of the raised band of stomach cilia, the solid arrow shows the direction of movement of particles in the higher focal levels whereas the dotted arrow shows the movement in the lower levels. *Cil. B.*, raised band of cilia; *Dig. Gl.*, digestive gland; *F.G.*, foregut; *H.G.*, hindgut; *R.M.*, right midgut diverticulum; *St.*, stomach.

The larval feeding mechanism of *Trinchesia aurantia* and *Eubranchus exiguus* in functional essentials differs not at all from that found in larvae of shell-type B, but significant differences are manifest in the arrangement of the visceral organs. Fig. 7 shows the larval gut of *Trinchesia aurantia*; it is obvious that the long axis of the stomach in this species lies along the antero-posterior axis of the larva, while in *Archidoris* (see Fig. 5) and all the other nudibranchs of shell-type B, it lies at right angles to this. The significance of this is not known; in any event the mechanism of food capture in *Trinchesia aurantia* and *Eubranchus exiguus* does not differ from the general pattern, nor could any difference in the treatment of ingested particles be detected. The absence of hyaline rods in the stomach wall of these two species, however, may be of importance.

DISCUSSION

There can be no doubt that nudibranch larvae feed during planktonic life. That this planktonic period is a short one is highly probable (Thorson, 1946) but to dismiss the group as of no importance in the plankton would be inaccurate. All British nudibranchs (leaving aside the Ascoglossa) have a planktonic larval phase and each individual adult produces very large numbers of eggs during the spawning season.

The mechanism of feeding was very similar in all the species investigated, some difference, however, existing between the orientation of the stomach of the larval shell-types B and C of Thorson (1946). Thorson's contention that nudibranch larvae with shells of type C are more fitted for planktonic life than those of type B finds no support from this investigation: larvae of the two types possess the same organs developed to the same degree.

The mechanism of particle capture in nudibranch larvae is similar in many respects to that described by Yonge (1926) for larvae of *Ostrea*, except that in nudibranchs the subvelar cilia are placed on a pair of prominent ridges. Movement of particles between the lumina of the visceral organs in larval nudibranchs is accomplished chiefly by ciliary means, assisted probably by the muscular movements which have been observed to occur; in *Ostrea* larvae, however, Millar (1955) states that particles pass into and out of the midgut diverticula chiefly or solely as the result of muscular action. The absence of the crystalline style and gastric shield and the unimportant role of the right midgut diverticulum are further differences from the larvae of lamellibranchs. It is possible, however, that the numerous hyaline rods in the nudibranch larval stomach wall (Fig. 6B, *Hy.Rods*), occupying a similar position in all those species in which it is present, serve a function similar to that of the style. The rotation of the particle mass against the site of these hyaline rods suggests strongly this interpretation of their function.

Cellular ingestion of food particles occurs solely in the digestive gland. In nudibranch larvae the right midgut diverticulum plays no part in the digestive process and the foregut and hindgut are concerned simply with the conduction of particles. The contents of the stomach and digestive gland in sections stain lightly with mucus stains and with haematoxylin, suggesting that intracellular digestion, which undoubtedly is the chief method of particle assimilation, may be aided by the production of extra-cellular enzymes. The presence of diffuse organic matter in the lumen of the gut is difficult to interpret otherwise.

The author is indebted to Mr J. S. Colman for helpful criticism and for the provision of laboratory facilities. The work was done while the author was the holder of a Leverhulme Fellowship in the University of Liverpool. The specimens of *Limapontia depressa* were most kindly brought by Mr A. Hopson from Flatford Mill, and those of *Alderia modesta* sent by Dr T. Gascoigne from Anglesey.

SUMMARY

The mechanism of food capture and the movement of food particles within the gut are described from observations on live and sectioned veliger larvae of the glossodorid nudibranch *Archidoris pseudoargus*.

Less detailed observations on the larvae of twelve other nudibranch species with shells of Thorson's type B, and of two species with shells of type C, showed similar mechanisms to exist in all.

Ingestion and assimilation of food particles occur solely in the left midgut diverticulum or digestive gland; some indirect evidence of the presence of extra-cellular enzymes is described.

REFERENCES

Barron, D. H., 1934. Amyl acetate: a useful solvent for embedding masses. *Anat. Rec.*, Vol. 59, pp. 1–3.

Carter, G. S., 1926. On the nervous control of the velar cilia of the nudibranch veliger. *Brit. J. exp. Biol.*, Vol. 4, pp. 1–26.

Millar, R. H., 1955. Notes on the mechanism of food movement in the gut of the larval oyster, *Ostrea edulis*. *Quart. J. micr. Sci.*, Vol. 96, pp. 539–44.

Pelseneer, P., 1911. Recherches sur l'embryologie des gastropodes. *Mém. Acad. R. Belg. Cl.Sci.*, Ser. II, 3, livr. 6, pp. 1–167.

Rasmussen, E., 1944. Faunistic and biological notes on marine invertebrates I. The eggs and larvae of *Brachystomia rissoides* (Hanl.), *Eulimella nitidissima* (Mont.), *Retusa truncatula* (Brug.) and *Embletonia pallida* (Alder & Hancock), (Gastropoda marina). *Vidensk. Medd. dansk naturh. Foren. Kbh., Bd.* 107, pp. 207–33.

—— 1951. Faunistic and biological notes on marine invertebrates. II. The eggs and larvae of some Danish marine gastropods. *Vidensk. Medd. dansk naturh. Foren. Kbh.*, Bd. 113, pp. 202–49.

Steedman, H. F., 1950. Alcian blue 8GS: a new stain for mucin. *Quart. J. micr. Sci.*, Vol. 91, pp. 477–9.

Thompson, T. E., 1958. The natural history, embryology, larval biology and post-larval development of *Adalaria proxima* (Alder & Hancock) (Gastropoda Opisthobranchia). *Phil. Trans.* B, Vol. 242, pp. 1–58.

Thorson, G., 1946. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund). *Medd. Komm. Havundersøg, Kbh.*, Ser. Plankt, Bd. 4, No. 1, 523 pp.

Vestergaard, K. & Thorson, G., 1938. Über den laich und die larven von *Duvaucelia plebeja*, *Polycera quadrilineata*, *Eubranchus pallidus* and *Limapontia capitata* (Gastropoda, Opisthobranchiata). *Zool. Anz.*, Bd. 124, pp. 129–38.

Winckworth, R., 1932. The British marine Mollusca. *J. Conchol.*, Vol. 19, pp. 211–52.

—— 1951. A list of the marine Mollusca of the British Isles: additions and corrections. *J. Conchol.*, Vol. 23, pp. 131–4.

Yonge, C. M., 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. *J. mar. biol. Ass. U.K.*, Vol. 14, pp. 295–386.

J. mar. biol. Ass. U.K. (1959) 38, 249–261
Printed in Great Britain

ECOLOGY OF THE GENUS *ACANTHOCHONDRIA* OAKLEY (COPEPODA PARASITICA)

By Z. Kabata
The Marine Laboratory, Aberdeen

(Text-figs. 1–4)

Among the parasites of the branchial chamber of the flatfishes of the Eastern North Atlantic, those belonging to the genus *Acanthochondria* (fam. Chondracanthidae, tribe Cyclopiformes) occupy a conspicuous place. Both their relatively large size and their abundance, render them easily noticeable even to a casual observer, in spite of the protective nature of their environment. It is a reflexion on the state of our knowledge of marine parasites that, notwithstanding their accessibility, very little is known about the animals of this genus.

The genus *Acanthochondria* was erected by Oakley (Leigh-Sharpe and Oakley, 1927) for the chondracanthids, which until then were included in *Chondracanthus* Delaroche, 1811, and which differed from the other members of that genus in the absence of the cephalic barbs and the dorsal and ventral processes. They were also characteristic in their preference for the flatfish hosts.

The workers who studied the family Chondracanthidae in the past were chiefly concerned with its taxonomy. A short résumé of the work on the family was given by Oakley (1930), who contributed greatly to our understanding of the relationships of the chondracanthids, but did not go beyond their systematics. Of the more recent workers Heegaard (1945, 1947) studied the development of *Acanthochondria*, and Debouteville (1951 *a*, *b*) the general biology of the chondracanthids by observation, among others, of living animals. Their work, however, was also intended to bring out the taxonomic and phylogenetic relationships, both within the family and within the Arthropoda as a phylum. Similar purposes were served by the work of Illg (1948).

It is intended in this paper to consider the genus *Acanthochondria* from the ecological point of view, by discussing the distribution, abundance and the details of the habitats of its members in the Eastern North Atlantic. The latter term is used here to cover three areas: the northern North Sea and the seas around the Faroe Islands and Iceland. The great majority of data obtained in the course of this work were collected by the Fishery Research Ships "Scotia" and "Explorer" of the Scottish Home Department's Marine Laboratory, Aberdeen. Some of the specimens of *A. cornuta* were collected by the Laboratory's earlier vessel "Goldseeker" in Scottish waters in 1921 and those of *A. solae* came from the collection of the late Dr T. Scott, now in the Royal Scottish Museum, Edinburgh.

ACANTHOCHONDRIAN FAUNA OF THE AREA

It is unfortunate that no agreement seems to exist at present as to the number and identity of the species of *Acanthochondria* occurring in the area under investigation. Apart from three undoubtedly valid species (*A. clavata*, *A. limandae* and *A. depressa*) there exist three more (*A. cornuta*, *A. flurae* and *A. solae*), the identity of which has been doubted by some authorities. Brian (1906) suggested that *A. flurae* and *A. solae* are merely varieties of *A. cornuta*. Hansen (1923) also regarded *A. cornuta* and *A. flurae* as being phenotypes of the same species. Hansen's opinion was accepted by Stephensen (1940) but not followed by Leigh-Sharpe & Oakley (1927), Oakley (1930) and Oorde-de Lint & Stekhoven (1936).

A. solae has not been found by the author. It is very rare in the area and will not be considered in detail in this paper. The morphology of the specimens from Dr T. Scott's collection was so distinct, that the author found it impossible to agree with Brian's opinion concerning this species. Brian's and Hansen's objections to the separation of *A. flurae* from *A. cornuta* cannot, however, be easily dismissed. Those who believe these two parasites to be distinct, regard the shape of the trunk as the main discriminant. The trunk of *A. cornuta* is taken to be long and slender, that of *A. flurae* short and squat. Another distinguishing feature used is that of specificity, *A. cornuta*, apparently occurring on the plaice, *Pleuronectes platessa* L., while *A. flurae* occurs on the long rough dab, *Drepanopsetta platessoides* Malm. According to Hansen, however, all the intermediate stages between the two typical shapes of the trunk existed and could be found on both flatfishes.

To check quantitatively the range of variation in the shape of the trunk of both parasites, the author compared the length: width ratios of 106 specimens collected from the long rough dab with those of 31 specimens from the plaice. The ratios of the former ranged from 1·9 to 3·3 (mean ratio 2·5) and those of the latter from 2·8 to 5·0 (mean ratio 3·6). Statistical analysis shows the significance of the difference between these groups of ratios to be at 0·001 level. It would appear, therefore, that in spite of some overlap of ratios the retention of the specific status for both these parasites is correct. The position is made complicated, however, by the existence of a population of *Acanthochondria*, superficially resembling those on the plaice (*cornuta* type), on the witch, *Glyptocephalus cynoglossus* (L). This flatfish has been previously recorded as a host of *A. flurae*. The examination of 10 specimens from it has shown their length: width ratios to range between 3·8 and 6·0 (mean ratio 4·8). The level of significance of the difference between the parasites of the plaice and those of the witch is also at 0·001 level. We have thus three significantly different populations, one (from the long rough dab) of *flurae* type and two of *cornuta* type.

The mouthparts of *Acanthochondria* have been studied by Oakley (1930) and were used by him as diagnostic features in identifying various species of the genus. His valuable work is now in some respects outdated and the mouthparts require re-examination. Because of the scarcity of material, Oakley (personal communication) was unable to dissect his specimens and had to rely on the examination of whole mounts. Examined from one aspect only, the shape of the mandibular palp,* as shown by Oakley, bears only a general resemblance to this appendage as examined by the author. In our present state of knowledge this structure cannot be used in diagnosing specific differences.

TABLE 1. SPECIES OF *ACANTHOCHONDRIA* OCCURRING IN THE EASTERN NORTH ATLANTIC AND THEIR HOSTS

	Host species	
Species of *Acanthochondria*	According to previous reports	As found by the author
A. cornuta (O. F. Müller, 1777) (Fig. 1)	*Platichthys flesus* (L.), *Pleuronectes platessa* L., *Scophthalmus maximus* (L.), *Lepidorhombus whiff-iagonis* (Walbaum)	*Pleuronectes platessa* L., *Glyptocephalus cynoglossus* (L.)
A. solae (Krøyer, 1838)	*Solea vulgaris* Day, *Platichthys flesus* (L.), *Pleuronectes platessa* L.	
A. flurae (Krøyer, 1863) (Fig. 1)	*Drepanopsetta platessoides* Gill, *Glyptocephalus cynoglossus* (L.)	*Drepanopsetta platessoides* Gill, *Lepidorhombus whiff-iagonis* (Walbaum), *Platichthys flesus* (L.)
A. limandae (Krøyer, 1863) (Fig. 1)	*Limanda limanda* (L.) *Platichthys flesus* (L.)	*Limanda limanda* (L.)
A. clavata (Basset-Smith, 1896) (Fig. 1)	*Microstomus kitt* (Walbaum)	*Microstomus kitt* (Walbaum)
A. depressa (T. Scott, 1905)	*Platichthys flesus* (L.)	

No final decision can as yet be reached on morphological grounds as to the distinctness of the two species. Ecological differences, however, encountered by the author in his study of *Acanthochondria*, tended to separate the parasites of the long rough dab on the one hand and those of the plaice and the witch on the other. In view of those differences the author decided to treat them as distinct and regard those from the long rough dab as *A. flurae* and those from other two hosts as *A. cornuta*.

Bearing the foregoing remarks in mind we can now postulate that six species of *Acanthochondria* occur in the investigated area. They are listed in Table 1, which shows also their host species, both as they are recorded in literature and as found by the author.

---

* Regarded by Heegaard (1947) as the first maxilla.

Numerous records of the occurrence of *Acanthochondria* in the eastern North Atlantic have been quoted by Oorde de Lint & Stekhoven (1936). According to these records, *A. cornuta* (Fig. 1 A) occurs in various parts of the North Sea and at Faroe and Iceland. *A. clavata* (Fig. 1 D) is said to occur in the North Sea (Firth of Forth), Firth of Clyde, and at Faroe. *A. depressa* occurs in the North Sea, *A. flurae* (Fig. 1 B) on the Scottish coast and at Faroe and Iceland, *A. limandae* (Fig. 1 C) in Moray Firth and Firth of Clyde and at Faroes, *A. solae* in the North Sea.

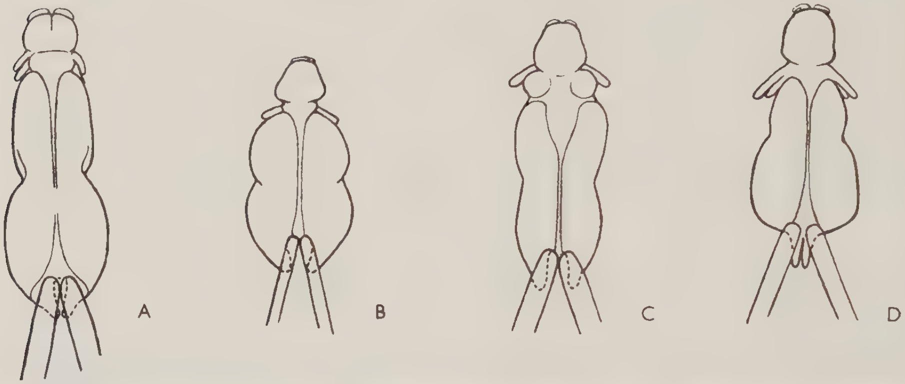


Fig. 1. General appearance of (A), *Acanthochondria cornuta*, (B) *A. flurae*, (C) *A. limandae* and (D) *A. clavata*.

To study the incidence of *Acanthochondria* the author examined 3961 flatfishes: 2553 from the North Sea, 419 from Faroe and 990 from Iceland. No specimens were found of *A. solae* or *A. depressa*. The latter species infests the flounder, *Platichthys flesus* (L.), and this fish was not extensively examined by the author, as the majority of the samples were collected from the open sea. The remaining four species, according to the data collected, are distributed quite widely throughout the localities inhabited by the host populations and show no distinct pattern of distribution within the separate areas.

The genus *Acanthochondria* shows a decrease in the number of species in the northerly direction. Six species are present in the North Sea, four off the Faroes and only two off Iceland. *A. depressa* and *A. solae* are absent from the acanthochondrian fauna of Faroes and Iceland. Only *A. flurae* and *A. cornuta* have been found in Icelandic waters. The explanation of this pattern in terms of limits to the distribution of the host populations is not quite satisfactory. For example, the lemon sole, *Microstomus kitt* (Walbaum), is found throughout the entire area, but is not infested at Iceland.

Consideration of distribution of *Acanthochondria* invites speculation on the way in which this genus spread throughout the area. The spread seems to have proceeded northwards from a southern centre, probably the environmental conditions acting as the chief limiting factors. A similar way of colonizing the northern grounds has been suggested by the author (1957) for the genus *Lernaeocera*.

ABUNDANCE

The abundance of *Acanthochondria* varies quite considerably both from species to species and from area to area.

In the North Sea *A. flurae* and *A. clavata* infest more than 20% of their host populations, *A. cornuta* 11% and *A. limandae* 2% (Table 2). A similar relative abundance is shown by the two species present at Iceland, *A. flurae* and *A. cornuta* (Table 3).

TABLE 2. ABUNDANCE OF *ACANTHOCHONDRIA* IN THE NORTHERN NORTH SEA

Flatfish species	No. examined	No. infested	% infested	Species of parasite
Pleuronectes platessa	155	8	5·2	*A. cornuta*
Platichthys flesus	19	2	10·5	*A. flurae*
Microstomus kitt	407	96	23·6	*A. clavata*
Drepanopsetta platessoides	1001	202	20·2	*A. flurae*
Limanda limanda	704	17	2·4	*A. limandae*
Glyptocephalus cynoglossus	98	11	11·2	*A. cornuta*
Lepidorhombus whiff-iagonis	159	—	—	—
Hippoglossus hippoglossus	9	—	—	—

TABLE 3. ABUNDANCE OF *ACANTHOCHONDRIA* OFF ICELAND

Flatfish species	No. examined	No. infested	% infested	Species of parasite
Pleuronectes platessa	118	9	7·6	*A. cornuta*
Microstomus kitt	131	—	—	—
Drepanopsetta platessoides	410	96	23·2	*A. flurae*
Limanda limanda	105	—	—	—
Glyptocephalus cynoglossus	101	3	2·9	*A. cornuta*
Lepidorhombus whiff-iagonis	96	1	1·0	*A. flurae*
Hippoglossus hippoglossus	29	—	—	—

TABLE 4. ABUNDANCE OF *ACANTHOCHONDRIA* OFF THE FAROES

Flatfish species	No. examined	No. infested	% infested	Species of parasite
Pleuronectes platessa	26	10	38·5	*A. cornuta*
Microstomus kitt	150	59	39·3	*A. clavata*
Drepanopsetta platessoides	62	11	17·7	*A. flurae*
Limanda limanda	70	25	35·7	*A. limandae*
Hippoglossus hippoglossus	111	—	—	—

The abundance of acanthochondrians off the Faroes is, however, quite different (Table 4). With the important exception of *A. flurae*, which shows an infestation incidence of the same order as in the other areas, all the three remaining species are much more abundant. Most surprising is the increase in abundance in comparison with the North Sea of *A. limandae* and *A. cornuta*, both of which infest over 30% of their host populations.

The abundance of a parasite cannot be fully measured by the percentage of the host population infested. It is also important to consider the numbers of parasites on an individual host. The details of this aspect of abundance are shown in Table 5.

TABLE 5. NUMBERS OF *ACANTHOCHONDRIA* ON AN INDIVIDUAL FISH

	North Sea		Faroes		Iceland	
Species	Nos. on one fish	Host/Parasite	Nos. on one fish	Host/Parasite	Nos. on one fish	Host/Parasite
A. flurae	1–5	3·5	1–3	4·1	1–7	2·7
A. cornuta (witch)	1–5	5·3	—	—	1–2	25·2
A. cornuta (plaice)	1–2	16·6	1–6	1·3	1	10·7
A. clavata	1–9	2·3	1–23	0·98	—	—
A. limandae	1–2	26·1	1–2	2·0	—	—

HABITAT

The members of the genus *Acanthochondria* are described by various authors as parasites of the gill cavity, of the gill arches or the gills. No study has been made, to the knowledge of the author, to determine the exact character of their habitat. Even less is known of the possible differences of the habitat between the species.

The numbers of both parasites and hosts studied in the course of this work have been sufficiently large to learn something of the habitats of *Acanthochondria*. No two species of this genus were identical in the manner of distribution within the gill cavity of their corresponding hosts. There existed also differences in the manner of association of *A. cornuta* with its two different hosts.

To examine habitats available to *Acanthochondria* on potential hosts, the opercular cavities of seven flatfish species were examined, by dissection and by making casts of subopercular spaces in latex rubber. The latter method is especially useful, as the shape of the cast remains unaffected by any strains during its removal.

The study of the respiratory apparatus of flatfishes has shown differences between the species, which may be said to form a range between two extremes, referred to here as "open" and "closed" types. The difference between the two types depends on the degree of development of the branchiostegal membranes, their relationship in space to the gills, on the amount of free space under the opercula and on the size and the shape of the mouth gape. The most typical representatives of the "open" type are the halibut, *Hippoglossus hippoglossus* (L.) and the megrim, *Lepidorhombus whiff-iagonis*. It will be seen from Fig. 2A that the branchiostegal membrane of the megrim is narrow, forming only a shallow pocket with the operculum, along the posterior border of which it is missing altogether. With the operculum *in situ* the inner rim of the membrane is situated at some distance from the tips of the gill filaments, especially in the posterior part of the cavity. The gills, in other words, are not enclosed in the branchiostegal pocket. The membranes of the opposite opercula are not fused to any great extent. The gape of the mouth is wide. It appears that this type of the respiratory apparatus is associated with active mode of life, since the halibut shows an essentially similar type of the gill cavity. These two fishes are not infested by *Acanthochondria* (149 of the examined halibut were found to be free of infestation and on 254 megrims only one specimen of *A. flurae* was found attached to the gill raker, a quite atypical habitat).

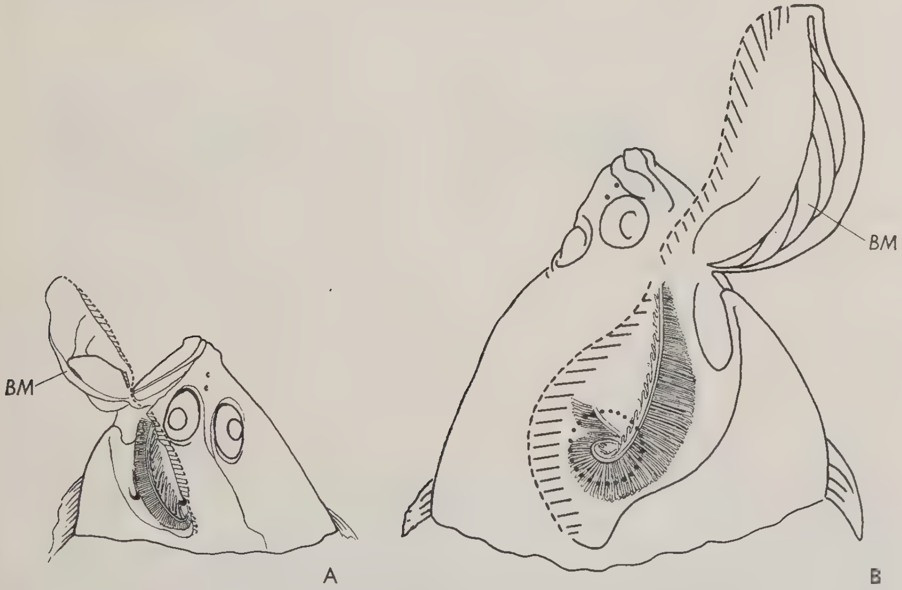


Fig. 2. Heads of (A) megrim and (B) plaice, with operculum dissected away to show spatial relations between various elements of the respiratory mechanism. Area of attachment of parasites indicated by dotted ring. *BM*, branchiostegal membrane.

Three further species of flatfish: the plaice, *Pleuronectes platessa* (Fig. 2B), the common dab, *Limanda limanda*, and the long rough dab, *Drepanopsetta platessoides* (Fig. 3A) are also of the 'open' type, although more towards the other end of the range. Their branchiostegal membranes are better developed, the tips of the gill filaments being just within the branchiostegal pockets. The gapes of the mouths are smaller than in the two preceding species, but still relatively well developed. These fishes, as far as we know, differ among themselves to some extent in their modes of life, the long rough dab being predatory and most active of the three. Not enough is known about the differences between the other two species, but their inclusion into the same group is not contrary to anything we know about their lives.

Nearer still towards the 'closed' type is the witch, *Glyptocephalus cynoglossus*, in which the branchiostegal pocket is more strongly developed, the membranes enclosing more of the gills and fusing with each other for a longer distance than in the preceding species. The gape of the mouth is smaller also.

At the end of the range is the extreme example of the 'closed' type, the lemon sole *Microstomus kitt* (Fig. 3B). In this species the membranes are fused quite a long way, enclosing the urohyal element of the pectoral girdle. The membranes are well developed, although narrowing posteriorly, and extend right round the opercular opening. The tips of the gill filaments project almost to the apex of the branchiostegal pocket, largely filling it along most of its length. The amount of free space under the opercula is in this species smaller than in any other of the examined flatfishes. It is too small to allow preparation of a good latex cast, only a thin film of rubber forming over most of the area. The mouth gape is very small. The fish is also relatively less active than other flatfishes, as indicated by its diet.

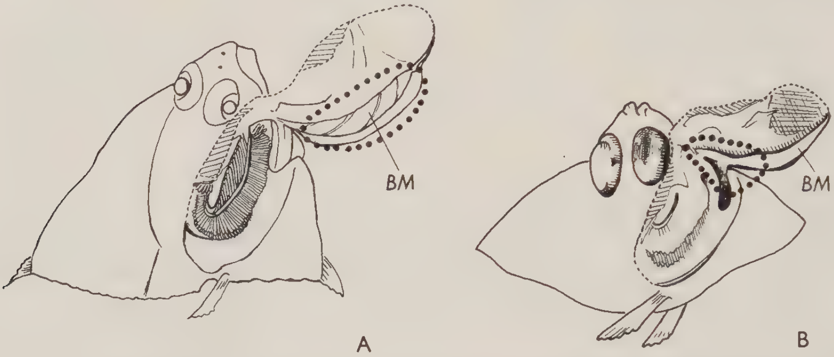


Fig. 3. Heads of (A) long rough dab and (B) lemon sole. Explanations as in Fig. 2.

As mentioned before, the first two species of the 'open' type are not infested by *Acanthochondria*. The next group of three shows two different ways of association with it. The first kind is illustrated by the distribution of *A. flurae* on the long rough dab (Fig. 4A). For the purpose of recording the distribution of the parasites within the opercular cavity, it was divided into four sections: three of roughly equal length along the edge of the operculum, the fourth comprising the urohyal. (The double line in the diagram indicates the extent of fusion of the branchiostegal membranes.) It will be seen that the great majority of the parasites (a sample of 331 *A. flurae*) are attached to the two posterior sectors of the opercula, all being within the branchiostegal pocket. Relatively few are found in the anterior sector of the opercula, where the membranes are fused. About one-third of the total number are attached to the urohyal. There is no significant difference between the two sides of the gill cavity, the ocular side harbouring 44·4% and the blind side 55·6% of the parasites.

The distribution of the parasites on the other two members of this 'open' group is entirely different. *A. cornuta* on the plaice is found in a very definitely circumscribed and small locality (Fig. 2B). It is attached usually in the narrow space between the end of the first branchial arch and the pseudobranch. Only very few exceptions were found in the anterior end of the opercular cavity, all firmly wedged between the bases of the branchial arches. *A. limandae* on the common dab is found only in the latter place. No parasites were found in the branchiostegal pocket of either plaice or common dab.

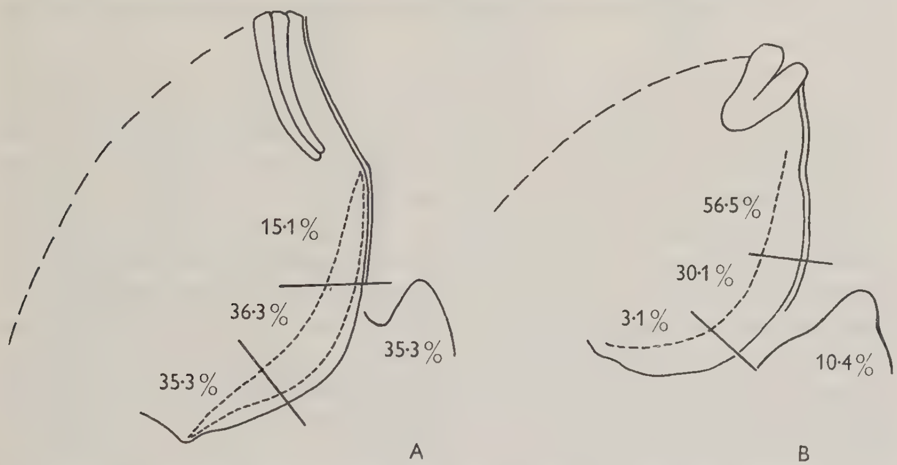


Fig. 4. Diagrams of the heads of (A) long rough dab and (B) lemon sole, showing the distribution of *Acanthochondria* in the opercular cavity.

The witch, more closely resembling the 'closed' type of the opercular structure is also infested with *A. cornuta*, which is so strikingly limited in the distribution on the plaice. Only few parasites were found on the witch, but those found were distributed along the branchiostegal pocket, not showing special preference towards any particular sector. None were present in the cleft between the pseudobranch and the base of the first gill arch and none either between the anterior bases of the arches.

The last of the hosts, the typically 'closed' type lemon sole, is heavily infested with *A. clavata*. The distribution of this species on the lemon sole is indicated by the dotted ring in Fig. 3B and diagram in Fig. 4B. The latter figure shows that more than a half of the sample of 193 parasites were distributed in the anterior sector of the branchiostegal pocket. The numbers of parasites in that pocket decreased posteriorly, only few being found in the posterior sector, where the membranes are most narrow. In this species there is also no appreciable difference between the ocular and the blind side of the cavity, the former carrying 52·3% and the latter 47% of the parasites.

DISCUSSION

The form of the host-parasite relationship is the outcome of the interaction of three factors: the host, the parasite and the external environment. All three factors contribute influences responsible for the final equilibrium. In the association between *Acanthochondria* and its flatfish hosts the operation of all these influences seems to be indicated by the facts related in the preceding sections of this paper.

The ecology of the host is undoubtedly responsible for the development of the specific relationship between it and the parasitic species. Unfortunately, any attempt to estimate the importance of this factor is made difficult by the gaps in our knowledge of the biology of the flatfishes. Data on distribution and abundance of *Acanthochondria* appear to indicate that the degree of infestation of the host species is related to its way of life, ecology and mechanics of respiration. In particular, the shape of the gill cavity must influence the distribution of the parasites. It is responsible for the distribution and the speed of the respiratory current within the cavity, both of which factors are likely to be of importance for the parasite. For the same reason the size and the shape of the mouth opening is likely to be important. More active species of the 'open' type of opercular structure and presumably having more abundant flow of water over the gills seem to be either free from infestation altogether or only slightly infested. The halibut and megrim are free, while the lemon sole is heavily infested. In apparent discord with this situation is the severe infestation of a host species of the 'open' type and of active mode of life, i.e. the long rough dab.

It is useful at this point to consider the role of the parasite in establishing host-parasite equilibrium. It seems that, on the whole, *Acanthochondria* favour habitats which are well protected and rather limited in the amount of free space. *A. cornuta* on the plaice appears to ignore the expansive areas of possible foothold offered by the broad branchiostegal membranes and lives only in the narrow gap, probably out of the way of the main respiratory current. The same species on the witch is found in the branchiostegal pocket, which in this fish is much narrower and has less free space, being filled by the tips of the gill filaments. It might be expected that the branchiostegal pocket of the witch has less flow of water and affords more protection than that of the plaice. *A. limandae*, found on the common dab, a fish of the same type as plaice, is also attached in a well protected and narrow space, this time between the bases of the gill arches. *A. clavata*, so abundant on the lemon sole, also appears to be most common in the best protected part of the branchiostegal pocket. Apart from the sensitivity to the conditions of the current, this might be related in some way to the well-developed tactile sense of the chondracanthids (Stekhoven, 1934; Goggio, 1927). It is not unlikely that a habitat affording optimal thigmotropic conditions might be chosen by a parasite which appears to possess some power to change the place of attachment (Stekhoven, 1934).

Structural similarity of various species of *Acanthochondria* is not necessarily paralleled by biological uniformity. It is probable that they differ among themselves both in demands put on their hosts and in the range of tolerance of the conditions of the external environment. One fact, pointing to possible biological differences is, for example, the difference in fecundity. According to the production of eggs per individual, the four species investigated in this paper can be divided into two groups:

	No. of eggs	Diameter of eggs (mm)
A. flurae	1,427	0·15
A. clavata	1,328	0·15
A. limandae	860	0·13
A. cornuta	759	0·14

The individuals sampled for egg counts were chosen so as to avoid possible variations due to the size of the animal, the locality and the season of the year. Statistical analysis of these numbers shows the difference between the two groups to be significant at the 0·001 level. The two less fecund species are also less abundant in two out of three areas examined. Both are also restricted to a very small locality in distribution on their hosts. Their eggs are slightly smaller, perhaps due to the lesser quantity of yolk contained and this might be of biological significance. It is also interesting that the only available specimen of the very rare *A. solae* had only 328 eggs, 0·12 mm in diameter, both values being lowest for any individual of any species examined.

Summing up the above considerations we might say that: (i) the more active the flatfish species, the less it is used as a host by *Acanthochondria*; (ii) this may be due to the parasites of the genus preferring environments well protected and restricted in space; (iii) the species which are less fecund, and more restricted in distribution on the host, tend to be less abundant. There are two important exceptions to these general principles. One active host of the 'open' type, the long rough dab, is heavily infested, the incidence of infestation being of the same order in all areas (Tables 2, 3, 4). At the same time the distribution of *A. flurae* on the long rough dab is consistent with the distribution of the space available within the gill cavity, irrespective of the degree of protection. It is suggested that this is possibly due to the lesser need of protection and wider range of tolerance of the environmental conditions on the part of *A. flurae*. Such tolerance would tend to eliminate to some extent the environmental influences from the interplay of the three factors involved in the host-parasite equilibrium, leaving it undisturbed over the large area. Some differences in the environmental conditions undoubtedly exist between the North Sea, Faroe and Iceland. The absence of *A. clavata* from Icelandic waters inhabited by an abundant population of the lemon sole, indicates the possibility of environmental limiting factors. In face of such possible differences *A. flurae* maintains a fairly constant degree of abundance in all areas.

In contrast to this uniformity of abundance of *A. flurae*, two other species, *A. limandae* and *A. cornuta*, are highly abundant on their respective hosts, the common dab and the plaice, at the Faroes (Table 3) but not at Iceland or in the North Sea, where they are less abundant than *A. flurae* and *A. clavata* (Tables 2 and 4). It is suggested that this might be due to their greater susceptibility to the environmental conditions, which favour their abundance at the Faroes but oppose it in the North Sea and at Iceland. That the environment at the Faroes is particularly favourable to *Acanthochondria* seems to be indicated by the fact that all the species of this genus, with the exception of *A. flurae*, are more abundant in that area than elsewhere.

All the biological differences between *A. flurae* and the remaining species, including *A. cornuta*, seem to lend support to those who believe in the distinctness of *A. flurae* from *A. cornuta*.

The author wishes to express his gratitude to Dr J. H. Fraser of the Marine Laboratory, Aberdeen, for his criticism of the manuscript and suggestions for its improvement.

SUMMARY

Four species of *Acanthochondria* are discussed in relation to their hosts, the flatfishes. The more active flatfishes, with less protected opercular cavities, appear to be less suitable as hosts for this genus of parasites.

A. flurae (found on the long rough dab) is equally abundant in the North Sea and at Faroe and Iceland. *A. clavata* (found on the lemon sole) is abundant in the North Sea, even more so at Faroe, but is absent from Icelandic waters. *A. limandae* (found on the common dab) is scarce in the North Sea, abundant at Faroe and absent from Iceland. *A. cornuta* (found on the plaice and the witch) is scarce in the North Sea and at Iceland, but very abundant at Faroe.

The four species differ in distribution on their respective hosts, all with the exception of *A. flurae* choosing well protected environments, which vary with the host. *A. flurae* appears to be less sensitive to the environment and directed in its choice more by the amount of space available for attachment. The abundance of the parasitic species in the area depends probably also on the environmental conditions.

The difference in biology between *A. flurae* and other species supports the opinion that it is a separate species.

REFERENCES

Brian, A., 1906. *Copepodi parassiti dei peschi d'Italia.* 187 pp. Genova.

Debouteville, C. D., 1951*a*. Présence de la sous-familie des *Pharonidae* Illg (Copepodes, *Chondracanthidae*) en Méditerranée et remarques sur la classification et la biologie des Chondracanthidae. *Arch. Zool. exp. gén.*, T. 87, pp. 139–55.

—— 1951*b*. Sur la biologie des Chondracanthidae (Crustacés Copépodes). *C.R. Acad. Sci., Paris*, T. 232, pp. 117–9.

Goggio, E., 1927. Richerche sulla vita, sulla conformazione esteriore e sulla struttura del *Chondracanthus lophii* Johnst. *Pubb. Staz. zool. Napoli*, Vol. 8, pp. 427–74.

Hansen, H. J., 1923. Crustacea Copepoda. II. Copepoda parasita and hemiparasita. *Dan. Ingolf-Exped.*, Vol. 3, (7), 92 pp.

Heegaard, P., 1945. Remarks on the phylogeny of the arthropods. *Ark. Zool.*, Vol. 37. No. 3, 15 pp.

—— 1947. Contribution to the phylogeny of the arthropods. Copepoda. *Spolia zool. Mus. Hauniensis*, Vol. 8, 236 pp.

Illg, P. L., 1948. *Pharodine*, a new subfamily of *Chondracanthidae* (Crustacea-Copepoda) and a description of *Pharodes biarensis* n.sp. from New Guinea. *J. Wash. Acad. Sci.*, Vol. 38, pp. 404–12.

Kabata, Z., 1957. *Lernaeocera obtusa* n.sp., a hitherto undescribed parasite of the haddock (*Gadus aeglefinus* L.). *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 569–92.

Leigh-Sharpe, W. H. & Oakley, C. L., 1927. *Lernentominae*, a new subfamily of *Chondracanthidae*, with a description of *Orialen triglae* (Blainville, 1822). *Parasitology*, Vol. 19, pp. 455–67.

Oakley, C. L., 1930. The Chondracanthidae (Crustacea: Copepoda); with a description of five new genera and one new species. *Parasitology*, Vol. 22, pp. 182–201.

Oorde de Lint, G. M. van & Stekhoven, J. H. S., 1936. Copepoda parasitica. *Tierw. N.- u. Ostsee*, Teil 10C, pp. 13–198 (Lief 31).

Stekhoven, J. H. S., 1934. Zur Sinnesphysiologie der parasitären Copepoden *Lepeophtheirus pectoralis* (O. F. Müller) und *Acanthochondria depressa* (T. Scott). *Z. Parasitenk.*, Bd. 7, pp. 336–62.

Stephensen, K., 1940. Parasitic and semiparasitic Copepoda. *Zoology of Iceland*, Vol. 3, Pt. 34, 24 pp.

J. mar. biol. Ass. U.K. (1959) 38, 263–266
Printed in Great Britain

RESPIRATION IN *CHONDRACANTHUS ZEI* DE LA ROCHE (COPEPODA)

By S. KRISHNASWAMY[1]

Department of Zoology, University, Southampton

(Text figs. 1–3)

In spite of the keen interest evinced in the taxonomy of the parasitic copepods, there is little information on their metabolic rate, though the respiration of free-living forms has been studied by such workers as Marshall & Orr (1955, 1957), Clarke & Bonnett (1939), Zeuthen (1947), Raymont & Gauld (1951), Gauld & Raymont (1953) and Conover (1956). Since the rate of oxygen consumption is a reflexion of the metabolic rate of an animal, it was felt that a study of the rate of oxygen consumption of a copepod parasite would be of interest. *Chondracanthus zei* De la Roche, 1811, is easily available and large.

C. zei is found on the gills and gill-arches of *Zeus faber* and is 'almost always present' (Oakley, 1930). Usually two to three parasites are found in each fish. It is a relatively large parasite, about 1·3 cm, and weighing up to 200 mg. It shows little movement in life except for occasional twitches, and is so deeply embedded in the tissue as to form a deep wound.

MATERIAL AND METHODS

The work was carried out at the Marine Biological Laboratory at Plymouth. The respiratory rate was followed in a Warburg manometer. Two to four animals, depending on their size, were used in each flask with 3 ml. of sea water; the CO_2 was absorbed by a roll of starch-free filter-paper dipping into 0·2 ml. of 20% KOH contained in the central wall of the flask. The manometers were shaken at sixty oscillations per minute. It was found that shaking did not damage the animal nor apparently in any way depress the respiratory rate. Pressures were corrected every 15 min and each experiment lasted for 3 h approximately. In all thirty-seven experiments were performed, but on occasions the same animals were used in more than one experiment owing to the difficulty in procuring enough material. All the experiments were carried out at 20° C. After each experiment, the animals were transferred on to filter-paper and weighed, and were then dried to constant weight in an oven at 100° C.

RESULTS AND DISCUSSION

The overall respiration rate varied from 19 to 30 μl. per copepod per hour. The same results expressed as oxygen consumed per mg dry weight per hour varied from 0·19 to 1·8 μl. depending on the size of the animal. There is a wide scatter (Fig. 1), especially in the smaller animals (50–60 mg range), which may partly be due to individual differences in respiratory rate. Nevertheless, the rate of oxygen consumption evidently shows a fall with increase in weight as has been shown in many poikilotherms by Zeuthen (1947). It is interesting to find a similar relationship for parasitic forms also. Von Brand (1953) mentions a similar trend in many endoparasitic animals, but unfortunately no information is available for comparison on external parasites.

---

[1] Colombo Plan Research Fellow.

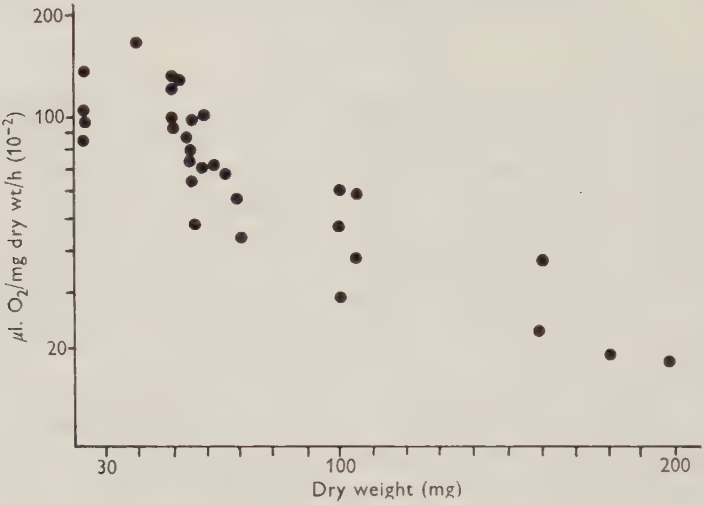


Fig. 1. Variations in the rate of oxygen consumption with body weight.

Marshall & Orr (1955, p. 115) observed that immediately after capture *Calanus* has a considerably higher respiration rate than some hours later, the fall being marked in the first few hours and not noticeable after about one day. Raymont & Gauld (1951) also found a similar fall in respiratory rate with time. During the present study the same trend was observed: the respiratory rate remained steady over a period of 3 h, but thereafter decreased. The results of a few experiments run continuously for 8 h clearly show that at the end the respiratory rate had fallen to a sixth of the initial rate (Fig. 2). This fall in respiratory rate probably explains much of the wide scatter in Fig. 1, since the same set of animals was used in more than one experiment. Experiments using the same animals on the second and third days also revealed a similar fall in respiratory rate (Fig. 3). However, on transfer to fresh sea water after each experiment, there appeared to be recovery in the rate, as evident from the gradual slope of Fig. 3. It is therefore probable that the decline in respiration was partly dependent on the oxygen tension in these long-term experiments.

The rate of oxygen uptake in *Chondracanthus* appears to be low as compared with a free-living copepod such as *Calanus finmarchicus*. A *Calanus* weighing 0·30 mg respires 0·75 µl. O_2, a relatively very large parasite weighing 200 mg requires only 0·19 µl./mg dry wt/h. This difference is presumably correlated with the parasite being less active.

A calculation shows that a *Chondracanthus* weighing 200 mg will require 3·85 % of its own body weight of food for its maintenance according to the O_2 uptake. In his review of Chondracanthidae Oakley (1930) states that these parasites probably live on a mixed diet of flesh and blood, and therefore the damage to the host must be considerable.

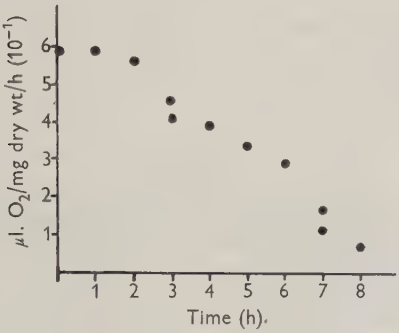


Fig. 2

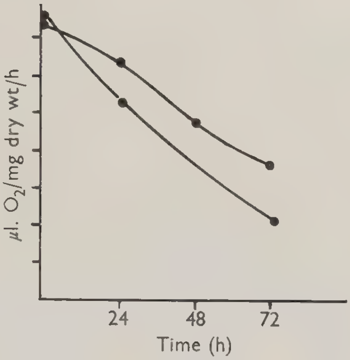


Fig. 3

Fig. 2. Fall in respiratory rate with time, in experiments run for 8 h continuously.

Fig. 3. Fall in respiratory rate with time, in experiments done on successive days.

In many animals a relationship has been established between surface area and respiration (Zeuthen, 1947, 1953). In a large parasite, like *Chondracanthus*, which lacks an efficient circulatory system, most of the oxygen required will have to be obtained by diffusion. Krogh (1941) pointed out that mere diffusion through the body surface may be insufficient to supply all the oxygen required in animals larger than 2 mm. It is therefore possible that the number of projections from the body surface helps to increase the surface area. Furthermore, *Chondracanthus* being located on the gill-arches is thereby exposed to a constant flow of fully oxygenated water so that the rate of diffusion of oxygen is maximal.

The author is indebted to Prof. J. E. G. Raymont of Southampton University for suggesting this problem and for helpful suggestions and criticisms. He also thanks the Director and Staff of the Marine Biological Laboratory, Plymouth, for the unfailing courtesy shown to him and for help and facilities. He is grateful to Dr E. D. S. Corner and Miss J. Lance for discussions and suggestions.

SUMMARY

The rate of oxygen consumption in *Chondracanthus zei* varied from 0·19 to 1·8 μl./mg dry wt/h depending on the body weight. There is a fall in respiratory rate with time. The metabolic rate as reflected by O_2 requirements shows that the animal may consume about 3·85% of its own body weight of food per day.

REFERENCES

Brand, Theodore von, 1952. *Chemical Physiology of Endoparasitic Animals.* 339 pp. New York: Academic Press.

Clarke, G. L. & Bonnet, D. D., 1939. The influence of temperature on the survival, growth and respiration of *Calanus finmarchicus. Biol. Bull., Woods Hole*, Vol. 76, pp. 370–83.

Conover, S. A. M., 1956. Biology of *Acartia clausi* and *A. tonsa. Bull. Bingham oceanogr. Coll.*, Vol. 15, pp. 157–233.

Dixon, M., 1951. *Manometric Methods.* Cambridge: University Press.

Gauld, D. T. & Raymont, J. E. G., 1953. The respiration of some planktonic copepods. II. Effect of temperature. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 447–60.

Krogh, A., 1941. *The Comparative Physiology of Respiratory Mechanisms.* 172 pp. Philadelphia: University Pennsylvania Press.

Marshall, S. M. & Orr, A. P., 1955. *The Biology of a Marine Copepod,* Calanus finmarchicus. 188 pp. Edinburgh: Oliver and Boyd.

—— 1957. A preliminary note on seasonal changes in respiration in copepods. *Année. Biol.*, Vol. 33, pp. 221–6.

—— 1958. On the biology of *Calanus finmarchicus.* X. Seasonal changes in oxygen consumption. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 459–72.

Oakley, C. L., 1930. The Chondracanthidae (Crustacea: Copepoda) with a description of five new genera and one new species. *Parasitology*, Vol. 22, pp. 182–201.

Raymont, J. E. G. & Gauld, D. T., 1951. Respiration of some planktonic copepods. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 681–93.

Scott, T. E. A., 1913. *The Parasitic Copepoda*, Vol. 1, 252 pp. London: Ray Society.

Zeuthen, E., 1947. Body size and metabolic rate in the animal kingdom. *C.R. Lab. Carlsberg*, Ser. Chim, T. 26, pp. 1–165.

—— 1953. Oxygen uptake as related to body size in organisms. *Quart. Rev. Biol.*, Vol. 28, pp. 1–12.

J. mar. biol. Ass. U.K. (1959) 38, 267–275
Printed in Great Britain

APPLICATION OF TOXIC AGENTS IN THE STUDY OF THE ECOLOGICAL RESISTANCE OF INTERTIDAL RED ALGAE

By A. D. Boney
Department of Chemistry and Biology, Plymouth Technical College
and E. D. S. Corner
The Plymouth Laboratory

(Text-figs. 1 and 2)

Biebl (1939*a*, *b*) has found that algae from various ecological groups show marked differences in their resistance to changes in the temperature of the surrounding sea water. He regards this temperature effect as an important factor influencing the distribution of different intertidal algae and points out that a change of only 2° C may prove critical with certain species. One of us (A.D.B.) has studied the resistance of sporelings of various common intertidal red algae to changes in environmental temperature and has obtained results similar to those of Biebl.

The present study is an attempt to assess the importance of the lipid moiety of the cell membrane in determining the degree of this resistance. Evidence of the amount of lipid material has been obtained by measuring the relative rates of penetration into the plants of substances of different lipid solubilities, it being assumed that if the cell membrane contains a large quantity of lipid material then substances of high lipid solubility will penetrate far more rapidly than those of low lipid solubility; whereas, if it has little then the relative rates of penetration of substances of high and of low lipid solubility will be much closer in value. Analysis of the contents of the plant cells for traces of the penetrating substance was considered too complex a task; therefore poisons were used, for their rates of penetration can readily be estimated by examining the toxic effects of the compounds on the growth and viability of the test organisms. The poisons chosen were *n*-propylmercuric chloride ($n\text{-}C_3H_7HgCl$) and mercuric chloride, compounds which have markedly different lipid solubilities (Corner & Sparrow, 1957) and are toxic to red algal sporelings (Boney, Corner & Sparrow, 1959).

In general, the results obtained support the view expressed by Blinks (1951) that lipids do, in fact, play an important role in determining the resistances of intertidal red algae to increased sea-water temperatures; moreover, the present findings indicate that these lipids are located in the cell membrane.

METHODS

Test species

The plants used were (1) *Polysiphonia lanosa* (L.) Tandy; (2) *Plumaria elegans* (Bonnem.) Schm.; (3) *Spermothamnion repens* (Dillw.) K. Rosenv.; (4) *Ceramium flabelligerum* J. Ag.; (5) *Ceramium pedicellatum* (Duby) J. Ag.; (6) *Antithamnion plumula* (Ellis) Thur. *var. plumula.* Most of these were collected from Church Reef, Wembury, where their shore habitats cover a fairly wide range. Thus, (1) grows on *Ascophyllum nodosum* (L.) Le Jol.; (2) and (3) are found in the subflora below *Ascophyllum*; (4) grows prolifically on *Laurencia pinnatifida* (Huds.) Lamour. in the landward crevices and overhangs from just above M.T.L. down to M.L.W.N.T.; (5) is found in tide pools and is particularly abundant at M.T.L.; (6) is generally sublittoral but, for the present study, spores were obtained from a persistent growth of the plant in the 'Drake's Island' tank at the Plymouth Laboratory.

Settlement of spores

Tufts of each plant, bearing tetrasporangia, were floated on to glass slides lying in an enamelled dish containing filtered sea water from outside Plymouth breakwater. The fruiting tufts were left overnight and the spores released were allowed to remain undisturbed for 2 days at 16° C before being used in the experiments. Usually, settlements of 150–200 sporelings were obtained on each slide.

Toxicity studies

Stock solutions of n-C_3H_7HgCl (5 mg Hg/l.) and $HgCl_2$ (50 mg Hg/l.) in sea water were prepared freshly for each experiment.

The methods used were those described by Boney *et al.* (1959) for experiments with sporelings of *Plumaria elegans.*

Estimations of lipid content

A known dry weight of the plant under test was extracted with absolute ethanol by maceration in a Waring blendor. The alcohol extract was then evaporated to dryness and the residue was extracted with warm petroleum ether (b.p. 40–60° C). The quantity of lipid material was then estimated by evaporating off the petroleum ether and weighing the dried residue.

RESULTS

Resistance to increased sea water temperatures

Estimates were made of the times for which 50% of a crop of sporelings of each plant survived in sea water at different temperatures. The results are shown in Table 1, from which it will be seen that sporelings of plants that grow in intertidal habitats where there is some exposure to air show a greater susceptibility to increased water temperatures, the one exception being *Ceramium flabelligerum*. Table 1 also includes the critical temperatures of the plants and these results correspond with those of Biebl (1939*a*) in that only three intertidal plants could tolerate 35° C and all were killed at 42° C.

TABLE 1. VIABILITIES OF SPORELINGS OF VARIOUS INTERTIDAL RED ALGAE IMMERSED IN SEA WATER AT DIFFERENT TEMPERATURES

Time (h) for which 50 % of sporelings survived

	Species readily withstanding exposure to air				Species with low resistance to exposure to air	
Temp (° C)	*Plumaria elegans*	*Polysiphonia lanosa*	*Spermothamnion repens*	*Ceramium flabelligerum*	*Ceramium pedicellatum*	*Antithamnion plumula*
25	32	—	—	—	—	—
27	17·5	27·5	37	—	—	—
30	0·33	10	13	36	32	32
32	0·25	0·49	—	8	—	15
34	0·08	0·16	1·0	—	2·5	2·0
36	—	—	0·50	1·0	1·5	1·0
38	—	—	—	0·16	—	—
40	—	—	—	0·08	0·08	0·08
42	0	0	0	0	0	0

Lipid contents of selected species

Experiments were carried out to test if the lipid contents of selected algae showed any marked differences, and if these differences correlated with corresponding ecological resistances. The results of this study are shown in Table 2 and demonstrate that although the total lipid contents of the three plants are very low, nevertheless the species which have a higher lipid content are those which are more susceptible to increased water temperature. However, these results did not give any indication of the distribution of lipid material within the plant cells, and to investigate this point experiments with poisons were made.

TABLE 2. TOTAL LIPID CONTENTS OF CERTAIN INTERTIDAL RED ALGAE

Species	Lipid content as % dry wt.
Plumaria elegans	0·91
Polysiphonia lanosa	0·55
Ceramium flabelligerum	0·34

Resistance to poisons

The toxicities of $HgCl_2$ and $n\text{-}C_3H_7HgCl$ were compared with 6 species of algae as the test organisms. The results are shown in Table 3, from which it will be seen that a very high ratio of LD_{50} values was obtained with those species that are most susceptible to increased sea-water temperatures (1, 2 and 3). The findings are therefore consistent with the view that these species possess a considerable amount of lipid material in the cell membrane.

TABLE 3. RELATIVE TOXICITIES OF N-C_3H_7HgCl AND $HgCl_2$ TO SPORELINGS OF VARIOUS INTERTIDAL RED ALGAE

Species	Low resistance to raised sea-water temperature			High resistance to raised sea-water temperature		
	(1) *Plumaria elegans*	(2) *Polysiphonia lanosa*	(3) *Spermothamnion repens*	(4) *Antithamnion plumula*	(5) *Ceramium flabelligerum*	(6) *Ceramium pedicellatum*
LD_{50} in $HgCl_2$ (mg Hg/l.)	6·7	8·0	3·0	5·0	3·2	4·2
LD_{50} in n-C_3H_7HgCl (mg Hg/l.)	0·021	0·030	0·0125	0·045	0·080	0·15
Ratio of above	319	266	240	111	40	28

Effect of temperature

It seemed probable that the lipid moiety of the cell membrane would be so distorted by increased temperature as to become more readily penetrable. For this reason a study was made at different temperatures of the effects of a poison of low lipid solubility on *Antithamnion* and *Plumaria*, species with markedly different resistances to temperature changes. The results obtained (see Table 4) showed that the enhanced toxicity of $HgCl_2$ conferred by increased temperature was far more marked in experiments with *Plumaria* than in those with *Antithamnion*, and these findings provided further evidence in support of the view that the lipid moiety of the plasma membrane is closely involved in temperature sensitivity.

TABLE 4. LD_{50} VALUES FOR $HgCl_2$ AT DIFFERENT TEMPERATURES

Sporelings immersed in the toxic solutions for 0·5 h at each temperature.

Temp °C	LD_{50} (mg Hg/l.)	
	Antithamnion plumula	*Plumaria elegans*
5	—	9·0
16	5·0	6·5
25	—	0·75
29	1·5	—
34	<0·5	—

Speed of toxic effect

It was considered likely that if the lipid moiety of the cell membrane was more developed in a species that was particularly temperature sensitive, this species would be only slowly penetrated by a poison of low lipid solubility. Accordingly, sporelings of several species were treated with $HgCl_2$ and examined for signs of toxic effects after 1 and 7 days. The results (Fig. 1) demonstrated that sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion* showed considerable resistance to poisoning by $HgCl_2$ when examined after 24 h, but that after 7 days the cumulative toxic effects of the poison had become

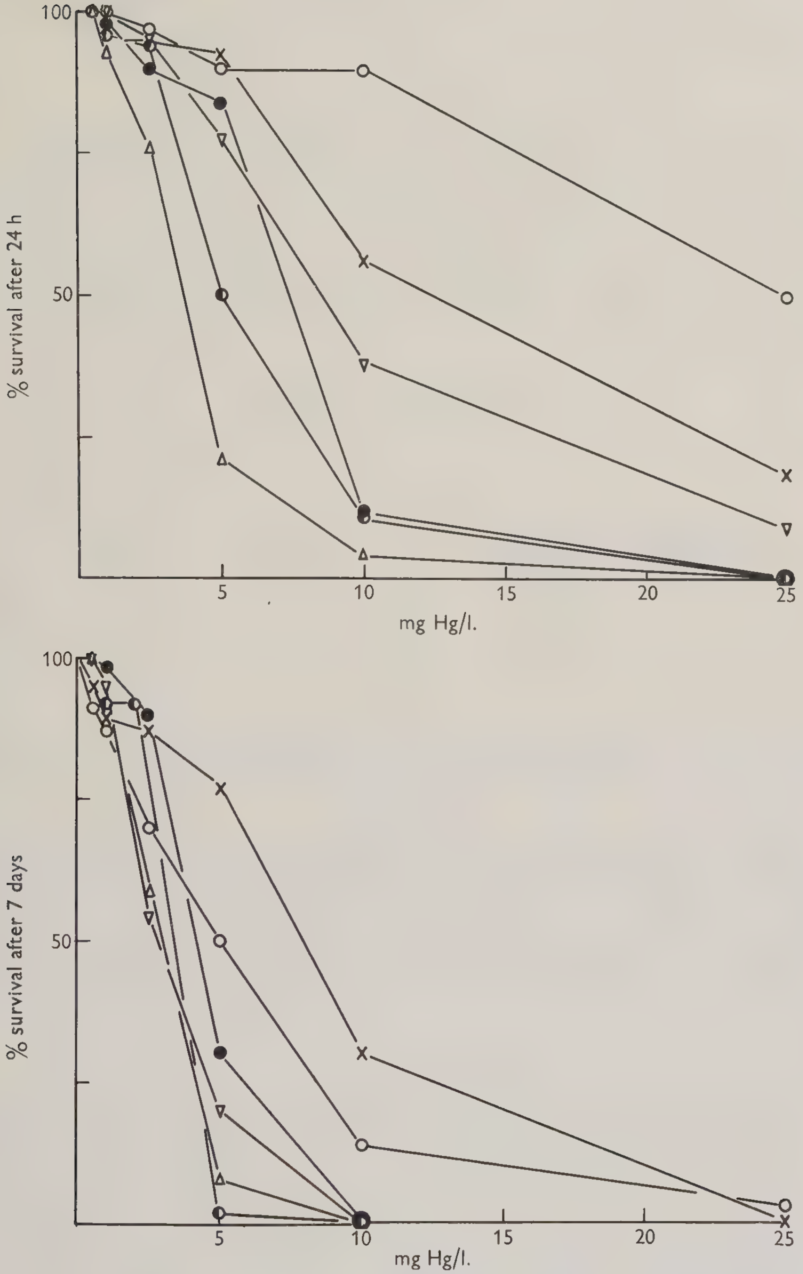


Fig. 1. Sporelings immersed 0·5 h in sea water containing various concentrations of mercury (as $HgCl_2$) then transferred to fresh sea water and examined 24 h (top figure) and 7 days (bottom figure) afterwards. ○—○, *Plumaria*; ×—×, *Polysiphonia*; ●—●, *Ceramium pedicellatum*; ◐—◐, *Antithamnion*; ▽—▽, *Spermothamnion*; △—△, *Ceramium flabelligerum*.

so marked as to make the order of resistance of all the test species approximately the same.

Distortion of the cell membrane with kerosene

Trim & Alexander (1949) have reported that non-toxic concentrations of kerosene may be used to distort the lipid barrier in certain cells which, as a result, become more easily penetrable. We tried this treatment, for it seemed likely that the most marked effect of kerosene would be found with those species which were thought to possess a well-developed lipid barrier. Preliminary experiments showed that immersion of the sporelings in sea water containing kerosene (0·1 ml./l.) for 5 min had no injurious effects on the young plants. In subsequent experiments, slides bearing the sporelings were immersed in the kerosene/sea water mixture, rinsed several times in filtered sea water and then transferred to toxic solutions of n-C_3H_7HgCl and $HgCl_2$. The results are summarized in Table 5, which shows that the most drastic reduction in the relative toxicities of the two poisons brought about by preliminary treatment of the test material with kerosene were observed in experiments with algal species that were assumed, from the results of previous studies, to possess considerable amounts of lipid material in the cell membrane.

TABLE 5. INFLUENCE OF KEROSENE ON THE RELATIVE TOXICITIES OF N-C_3H_7HgCl AND $HgCl_2$

Species numbered as in Table 3. The ratio given is the relative toxicity of the two poisons.

	Sporelings treated with kerosene			Sporelings untreated		
Species	LD_{50} in $HgCl_2$ (mg Hg/l.)	LD_{50} in n-C_3H_7HgCl (mg Hg/l.)	Ratio	LD_{50} in $HgCl_2$ (mg Hg/l.)	LD_{50} in n-C_3H_7HgCl (mg Hg/l.)	Ratio
1	4·3	0·029	148	6·7	0·021	319
2	3·0	0·080	38	6·0	0·023	260
3	1·0	0·031	32	3·0	0·0125	240
4	6·2	0·048	129	6·3	0·054	115
5	3·0	0·085	35	2·8	0·079	35
6	5·0	0·085	59	3·8	0·090	42

Experiments with 1:2-naphthoquinone

To gain further information about the importance of the lipid barrier, use was made of 1:2-naphthoquinone, a poison chemically unrelated to the mercury compounds, yet likely to penetrate cells very readily because of its high lipid solubility. Slides bearing the sporelings were immersed for 0·5 h in various concentrations of the poison in filtered sea water and the results obtained using three of the test species are summarized in Fig. 2, which shows the percentage of the sporelings that survived 24 h after their immersion in the poison. It will be seen that these results accord well with those obtained using $n\text{-}C_3H_7HgCl$, in that 1:2-naphthoquinone is more toxic to sporelings of the species known to be highly sensitive to increased water temperature.

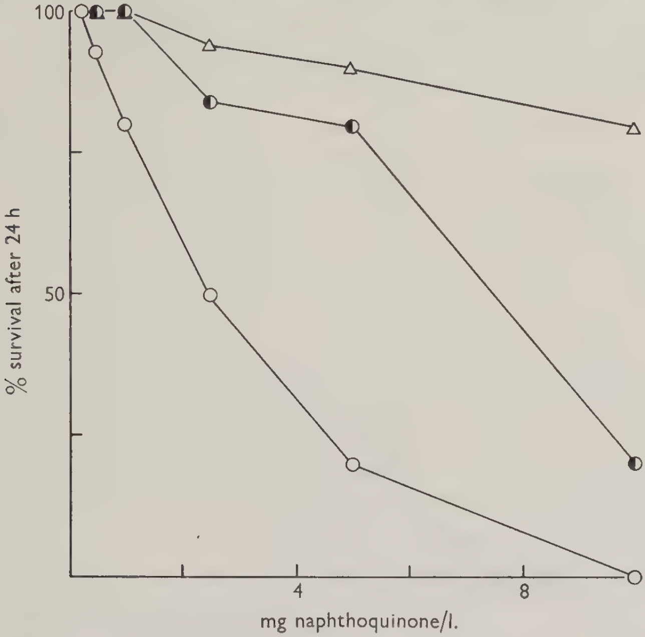


Fig. 2. Sporelings immersed 0·5 h in sea water containing various concentrations of 1:2-naphthoquinone then transferred to fresh sea water and examined 24 h later. △—△, *Ceramium flabelligerum*; ◐—◑, *Antithamnion*; ○—○, *Plumaria*.

DISCUSSION

Macpherson & Young (1949), examining the chemical composition of certain marine plants, found measurable quantities of lipid material in the red algae *Rhodymenia palmata* (3·78% of dry weight) and *Halosaccion ramentaceum* (2·82% of dry weight); but none in *Gigartina stellata*, *Chondrus crispus* and *Ahnfeltia plicata*. In the present study it was found that although three of the test species had only small lipid contents (0·34–0·9% of dry weight), nevertheless the alga most sensitive to temperature (*Plumaria elegans*) had the highest content and the species least sensitive to temperature (*Ceramium flabelligerum*) had the lowest. Furthermore, the use of toxic agents provided a good deal of evidence in support of the view that the cell membranes of species that were temperature-sensitive contained more lipid material than those of species that were temperature-resistant.

A complementary investigation of the resistances of sporelings of the test species to a number of environmental factors showed that sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion* were able to withstand exposure to air in the intertidal zones more readily than sporelings of *Ceramium pedi-*

cellatum and *Antithamnion*. Thus, sporelings of the first three species, when enclosed in a water film, were more resistant to prolonged exposure to air of 100% R.H., and survived for longer times in air of increased temperature. In addition, these same species showed a much more marked ability to withstand increased concentrations of salt in the surrounding sea water.

Sporelings of *Ceramium flabelligerum*, however, showed a marked resistance to both increased water temperature and to environmental factors arising from exposure to air: this distinct behaviour indicates that this species may be a 'southern' plant capable of showing a marked resistance to intertidal conditions in warmer climates. In the Plymouth area this plant is a common perennial that grows to maximum size during the summer, which is its main period of spore output. However, spore production is carried on throughout the winter by the small tufts that are left after the autumn defoliation. On northern shores of the British Isles the plant is rare and fruiting records are few. It may be said of the other five species examined that by their ecological and toxicological resistances, *Antithamnion* and *Ceramium pedicellatum* appear to be members of the 'southern component' of the algal flora, although they are much less resistant than *Ceramium flabelligerum* to factors arising from exposure to air: and that, by contrast, *Plumaria*, *Polysiphonia* and *Spermothamnion* are all 'northern plants'.

Although the findings suggest that sporelings of red algae which have a 'northern' range are sensitive to increased water temperature because their cell membranes are well endowed with lipid material, the further possibility cannot be excluded that temperature sensitivity may also be due to denaturing of proteins (*vide* Blinks, 1951). In fact it is possible that both these changes may occur and together account for subsequent inhibition of metabolic processes.

There seems no reason why the methods used in the present work should not be applied to many problems of ecological interest and further studies along these lines are planned with sporelings of several 'southern' and 'northern' species of red algae. For example, it would be of considerable interest to examine *Ceramium acanthonotum* Carm. ex Harv. and *Callithamnion arbuscula* (Dillw.) Lyngb. for, although these two species are clearly 'northern' plants, whereas the former also occurs on 'southern' shores the latter does not. In addition, similar studies on ecological resistances might be made using material collected from different localities within the geographical range of a particular species to supplement information about the form range of that species. For, as Parke (1953) has indicated, this is one of the most pressing problems in marine algal ecology.

One of us, A.D.B., is indebted to the Board of Governors of Plymouth Technical College for a grant enabling him to work at the Plymouth Laboratory. Both are grateful to Dr Mary Parke for valuable discussions.

SUMMARY

Sporelings of the intertidal red algae *Plumaria elegans*, *Polysiphonia lanosa* and *Spermothamnion repens* readily withstand exposure to air but have low resistance to increased sea-water temperature; those of *Antithamnion plumula* and *Ceramium pedicellatum* readily withstand increased sea-water temperature but have low resistance to exposure to air; those of *Ceramium flabelligerum* are resistant to both factors.

Poisons of high lipid solubility (n-C_3H_7HgCl and 1:2-naphthoquinone) are far more toxic than one of low lipid solubility ($HgCl_2$) to sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion*; but when *Antithamnion*, *Ceramium pedicellatum* and *Ceramium flabelligerum* are used as the test material the relative toxicities of the poisons are much closer in value.

The toxic effects of $HgCl_2$ to sporelings of *Plumaria* develop slowly at normal temperature and much more quickly when the temperature is raised. However, when sporelings of *Antithamnion* are used, toxic effects develop rapidly at both normal and raised temperatures.

The relative toxicities of n-C_3H_7HgCl and $HgCl_2$ to sporelings of *Plumaria*, *Polysiphonia* and *Spermothamnion* are markedly reduced when the test material is first treated with sub-toxic amounts of kerosene, a substance known to distort the lipid moiety of the cell membrane. However, when *Antithamnion*, *Ceramium pedicellatum* and *Ceramium flabelligerum* are used as the test material, the effect of the kerosene is negligible.

These results are consistent with the view that the proportion of lipid material present in the cell membrane is important in determining the susceptibility of an intertidal red alga to increased sea-water temperature and have been discussed with especial reference to the geographical distributions of the various species examined.

REFERENCES

BIEBL, R., 1939*a*. Über Temperaturresistenz von Meeresalgen verschiedener Klimazonen und verschieden tiefer Standorte. *Jb. wiss. Bot.*, Bd. 88, pp. 389–420.

—— 1939*b*. Protoplasmatische Oekologie der Meeresalgen. *Ber. dtsch. bot. Ges.*, Bd. 57, pp. (78)–(90).

BLINKS, L. R., 1951. Physiology and biochemistry of algae. *Manual of Phycology*. Waltham, Mass.

BONEY, A. D., CORNER, E. D. S. & SPARROW, B. W. P., 1959. The effects of various poisons on the growth and viability of sporelings of the red alga *Plumaria elegans* (Bonnem.) Schm. *Biochem. Pharmacol.* (in the Press).

CORNER, E. D. S. & SPARROW, B. W. P., 1957. The modes of action of toxic agents. II. Factors influencing the toxicities of mercury compounds to certain crustacea. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 459–72.

MACPHERSON, M. G. & YOUNG, E. G., 1949. The chemical composition of marine algae. *Canad. J. Res.*, Vol. 27, pp. 73–7.

PARKE, M., 1953. A preliminary check list of the British marine algae. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 497–520.

TRIM, A. R. & ALEXANDER, A. E., 1949. Surface activity and permeability as factors in drug action. *Symp. Soc. exp. Biol.*, No. 3, pp. 111–42.

J. mar. biol. Ass. U.K. (1959) 38, 277–290
Printed in Great Britain

ON THE STRUCTURE, BIOLOGY AND SYSTEMATIC POSITION OF *PHARUS LEGUMEN* (L.)

By C. M. Yonge, D.Sc., F.R.S.

University of Glasgow

(Text-Figs. 1–7)

The species with which this paper deals was originally included by Linné in the genus *Solen* and was there retained to be included under that name in the magnificently illustrated account of this genus by Deshayes (1845–48). It was later placed in a new genus *Ceratisolen* by Forbes & Hanley (1853) who considered that it formed 'the connecting link between the Solenidae and Solecurtidae'. This procedure was followed by Jeffreys (1865). More recently the Solecurtidae were shown by Graham (1934*b*) having 'regard to such features as shape, presence of a cruciform muscle and the structure of the foot, siphons, adductor and pedal muscles, supra-axial extension of the outer demibranch, palps, and style-sac and intestine', to belong to the Tellinacea, a view fully supported by this author (Yonge, 1949). Assigned later to the genus *Pharus* which has priority over *Ceratisolen*, this 'connecting link' of Forbes & Hanley continued to be associated with the Solenacea by Thiele (1935) in his great systematic account of the Mollusca.

Personal observations on the Tellinacea (Yonge, 1949) and later on *Siliqua patula* and other members of the Solenacea (Yonge, 1952) have indicated that *Pharus legumen* should also be transferred to the Tellinacea. This paper is primarily concerned with the reasons for this change in classification, and this has involved some description of structure and habits in this little known member of the British marine fauna. Nothing can supersede the magnificent plates in Deshayes (1845–48) but his account, although detailed, is not, by present standards, critical, while the very short description by Bloomer (1903) is of little value.

This work has been made possible largely through the kindness of Prof. E. W. Knight-Jones who, from Anglesey and later from the Swansea area, sent living specimens with information about the conditions under which they were found. Preserved specimens from Brittany were also kindly supplied by Mr N. A. Holme of the Plymouth Laboratory, while Prof. A. Graham and Dr V. Fretter of Reading have supplied preserved specimens and given information about habitat. Acknowledgements are also due to Dr H. F. Steedman and Dr G. Owen of this Department for technical help and also, in the case of Dr Owen, who is investigating allied problems, for much profitable discussion.

EXTERNAL APPEARANCE AND SHELL

As Deshayes and other early workers pointed out, *P. legumen* (Fig. 1) may be distinguished from other species of '*Solen*' by the situation of the ligament, in the middle instead of at the anterior end of the long flat dorsal surface of the shell. There is also greater lateral compression of the shell than in species of *Ensis* or *Solen* while the valves taper somewhat anteriorly (in his figure of the shell with foot and siphons projecting, Jeffreys (1865) shows this the wrong way round). The shell attains a length of up to some 10 cm and is about four times longer than it is deep. It is pale yellow and glossy with a characteristic difference in texture and with a paler colour over the posterior sector (indicated in Fig. 1). Hinge dentition, which may here be mentioned, consists of two cardinals in the left valve (see Fig. 2) and one on the right with, in each valve, a long and low anterior lateral and a short, projecting posterior lateral. There is nothing here of value in determining the systematic position of *Pharus*.

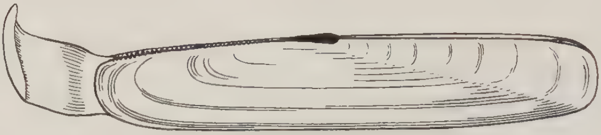


Fig. 1. *Pharus legumen*, viewed from left with foot partly extended. × $\frac{3}{4}$.

The elongated and very compressed foot (Figs. 1, 2 and 4) issues through a restricted anterior gape. It is truncated terminally and when observed in a bowl of water makes very sudden extensions to a length as great as that of the shell, at the same time opening out terminally. It is then as quickly withdrawn. It is clearly fully as effective in rapid vertical burrowing as the foot of *Ensis* or *Solen*, described by Fraenkel (1927), but it differs in being less bulbous terminally. Siphons longer than those of species of *Solen* or *Ensis* (although shorter than those of *Siliqua patula* (Yonge, 1952)) project posteriorly and to a length of 5 mm in an animal of shell length 3·9 cm. But, unlike all true members of the Solenacea, the inhalant and exhalant siphons are separate throughout (see Fig. 2) and are also, as shown later, fundamentally different in structure, a fact which initially indicated the affinity of this species to the Tellinacea.

Although first recognized as a British species by Martin Lister in the seventeenth century, *Pharus legumen* is relatively seldom encountered. It is a southern species (hence its description by Deshayes in an account of the marine molluscs of Algeria) and is here near the northern end of its range. It appears to be confined to the western end of the south coast, to the north coasts of Devon and Cornwall, to the shores of Wales and to all but the north coast of Ireland. It is not recorded from the Isle of Man or from the Clyde

Sea Area. It ranges in the south from Brittany, around the shores of Spain and Portugal into the Mediterranean where it appears to be common. In Great Britain it is certainly only abundant around the shores of Wales and there, judging from information received from Prof. Knight-Jones, is commoner along the south coast than around Anglesey. It lives in relatively clean sand, although with some admixture of organic silt. To quote from a letter from Prof. Knight-Jones dealing with the Swansea area: 'The sand at Oxwich Bay where *Pharus* occurs near here is fine and fairly clean. The water above it is generally very turbid and there is a lot of silt washing about on the surface of the sand.' At Roscoff (1951) it is stated as occurring 'A très basse mer, dans du sable plus ou moins pur'. It appears to inhabit a restricted zone on the margin of the lower shore and in the shallow sublittoral. Hence it can only be dug at low water of the greater spring tides while its capacity for rapid vertical burrowing makes it difficult to obtain by dredging which in any case is never easy in very shallow water. These restrictions, both in horizontal and vertical distribution around the shores of Great Britain, go far to explain the prevailing ignorance about this species.

PALLIAL STRUCTURES

Muscles. As shown in Fig. 2, the pallial (orbicular) muscles are attached at some distance from the margin of the shell. Both adductors, the anterior being much the longer, are extended longitudinally. The posterior adductor (*PA*), as recently indicated by Owen (1958) in work concerned partly with the Solenacea, does not represent the termination of the pallial muscles at that end. For functional reasons, as in the Solenacea and in other bivalves where the shell has elongated, the region of local muscular hypertrophy represented by the adductor has migrated posteriorly but pallial attachment continues, as indicated in Fig. 2, up to the posterior end of the ligament. The shell is strengthened in the region of the hinge by a ventrally extending rib, the anterior surface of which bounds the posterior margin of attachment of the anterior adductor. Such strengthening of a relatively delicate extended shell is not unusual, occurring also in *Siliqua patula*. It has certainly no systematic value. The siphonal retractors are well developed and the pallial sinus is correspondingly capacious.

Mantle folds and fusion. The mantle margins are united along the greater part of the ventral surface and, except for the openings of the siphons, posteriorly right round to the posterior end of the ligament. Fusion is of Type B (Yonge, 1957), i.e. of inner (muscular) folds of the mantle edge together with the inner surfaces of the middle lobes. The outer surfaces of the middle lobes form a narrow ridge (*MF*) between the outer lobes the innermost margin of which constitutes the periostracal groove (Fig. 3). The outer lobes are broad with periostracum extending for some distance within the

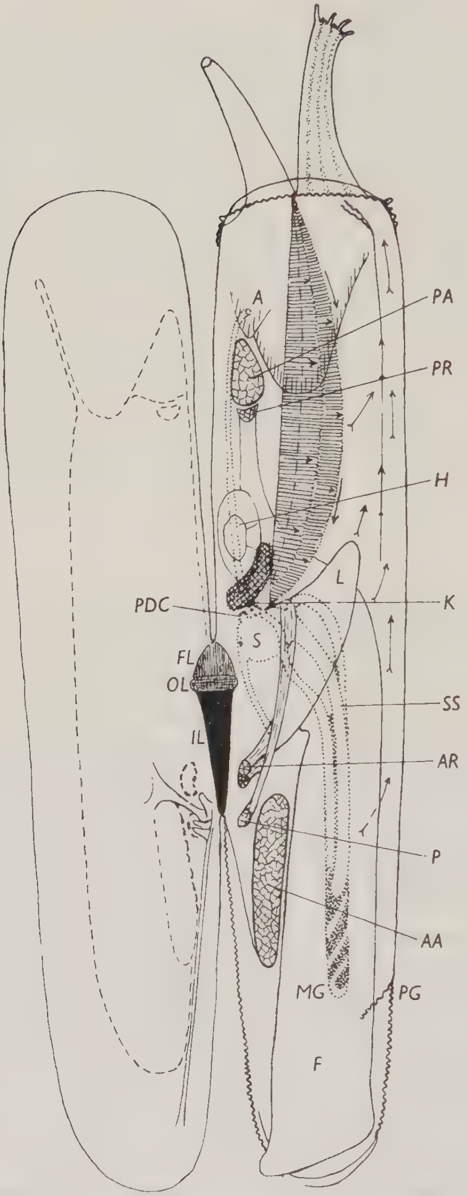


Fig. 2. *Pharus legumen*, animal lying in right valve with siphons extended, left valve turned back to show ligament, hinge teeth and muscle insertions (indicated by broken lines). × 1½. *A*, anus; *AA*, anterior adductor; *AR*, anterior pedal retractor; *F*, foot; *FL*, fusion layer of ligament; *H*, heart; *IL*, inner layer of ligament; *K*, kidney; *L*, labial palp; *MG*, coiled region of mid-gut extending into foot; *OL*, outer layer of ligament; *P*, pedal protractor; *PA*, posterior adductor; *PDC*, postero-dorsal caecum of stomach; *PG*, posterior end of pedal gape; *PR*, posterior pedal retractor; *S*, Stomach; *SS*, style-sac. Plain arrows indicate direction of food collecting currents, feathered arrows of rejection currents.

margin of the outer calcareous layer of the shell which is secreted by the outer surface of the outer fold (Yonge, 1957). On the dorsal surface anterior to the ligament, the middle mantle folds are nowhere united, their free margins being bounded by short tentacles as indicated in Figs. 1 and 2. But formation and anterior extension of the anterior adductor does represent extensive fusion of the inner mantle folds in this region. It should be noted that there is *no* cuticular fusion of the anterior regions of the mantle margins ventrally which is so characteristic a feature in the Solenacea where it occurs in *Solen*, *Ensis* and *Cultellus*; previously overlooked in *Siliqua patula* (Yonge, 1952), it has been found there by Owen (1959).

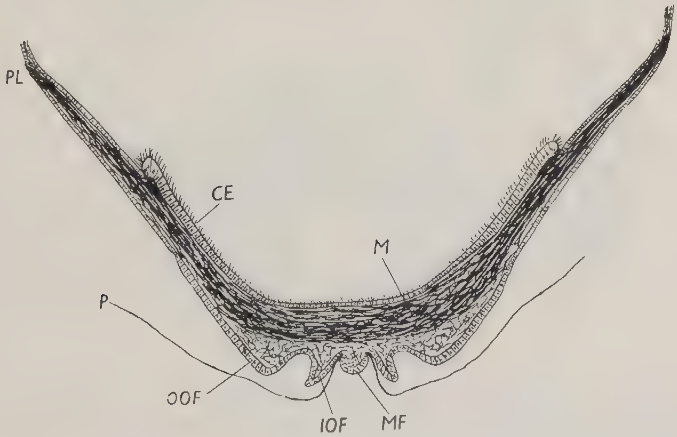


Fig. 3. *Pharus legumen*, transverse section through fused, mid-ventral region of mantle lobes posterior to palps (see Fig. 2). × 90. *CE*, ciliated epithelium; *IOF*, inner surface of outer fold of mantle margin (secretes periostracum); *M*, pallial muscle; *MF*, fused middle mantle folds; *OOF*, outer surface of outer mantle fold (secretes outer calcareous layer of shell); *P*, periostracum (other shell layers not shown); *PL*, pallial line.

The pedal gape extends from the anterior end of the ligament dorsally to a point some one-fifth of the distance back along the ventral surface. The single row of tentacles which fringe the middle mantle fold is well developed both ventrally and dorsally but anteriorly the tentacles are reduced over an area where the inner mantle folds (*IF*) increase greatly in depth and become frilled (Fig. 4). They form a pedal valve resembling that found in Solenacea such as *Ensis* and having presumably the same function, namely wiping the surface of the foot as it is being withdrawn.

Siphons. Separate to the base, these are formed exclusively from the inner folds of the mantle margin. The small middle folds with the adjacent periostracal groove (this representing the innermost margin of the outer fold) extend, as shown in Figs. 2 and 5, around the base of the siphons, uniting above and below. The exhalant siphon bears no marginal tentacles and curves slightly dorsalwards; the inhalant siphon is fringed with a series of eight large and sixteen very small tentacles, the latter arranged in pairs between the larger ones. The surface of this siphon carries eight pigmented lines which run to each of the pairs of small tentacles. In their complete separation and formation exclusively from inner mantle folds, these siphons are identical with those of the Tellinacea; siphons in the Solenacea are largely united, the common outer covering with its tentacles being formed from the middle mantle folds. Furthermore, in transverse section (Fig. 6), the siphons of *P. legumen* reveal the highly characteristic structure of the siphons of the Tellinacea as described and figured by Rawitz (1892), Hoffmann (1914), Graham (1934*b*), Yonge (1949) and Chapman & Newell (1956). Between the inner and the outer epithelia there lie alternating circular layers of collagen fibres (C_1–C_4)—originally described by all workers as circular muscle fibres but correctly interpreted by Chapman & Newell—and bundles of longitudinal muscle (L_1–L_3). There are four layers of the former and three of the latter, the outermost of these muscle layers being poorly developed. Radiating strands (*R*) of probably mixed muscle and collagen fibres traverse these concentric layers. In the zone occupied by the innermost muscle layer run the symmetrically disposed nerves (*N*), four in the exhalant and eight in the inhalant siphon. Haemocoels (*H*) occur on the inner side of the middle of the three muscle layers.

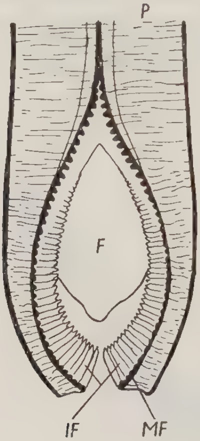


Fig. 4

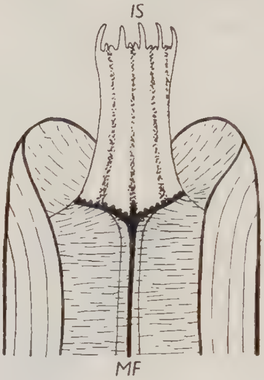


Fig. 5

Fig. 4. *Pharus legumen*, pedal gape viewed from ventral aspect. × 2. *F*, foot; *IF*, inner fold of mantle margin (hypertrophied to form pedal valve); *MF*, middle fold; *P*, periostracum.

Fig. 5. *Pharus legumen*, posterior end of shell with inhalant siphon viewed from ventral aspect. × 2. *IS*, inhalant siphon; *MF*, middle fold of mantle margin.

The entire picture is precisely that seen in cross-sections through the siphons of species of such characteristic genera of the Tellinacea as *Tellina*, *Macoma*, *Abra*, *Scrobicularia*, *Donax*, *Gari* and *Solecurtus* (Yonge, 1949). Only in the number of nerves is there some difference, this being six in species of all of the above genera where the inhalant siphon bears that number of fringing lobes. Rawitz, however, found that there were occasionally eight nerves in *Gari depressa* (presumably in association with that number of marginal lobes), while in *Tagelus dombeyi* the number is normally eight (Hoffmann, 1914). In the Solenacea the histological picture is completely different. Longitudinal, circular and radial muscles are all well developed but apart from a tendency (noted in sections of both *Ensis* and *Solen*) for longitudinal muscle to be most developed under the outer and inner epithelia, there is no trace of the organization into sharply defined zones so highly characteristic of the Tellinacea and associated, as demonstrated by Chapman & Newell, with an intrinsic and highly effective mechanism of extension and withdrawal.

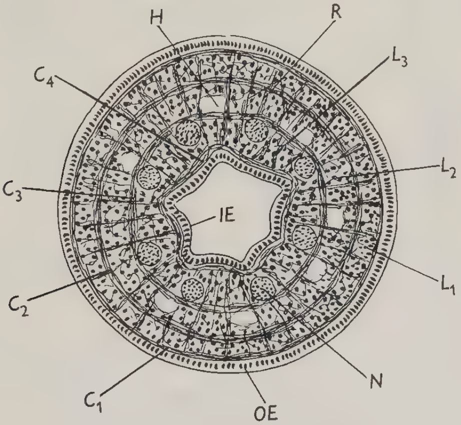


Fig. 6. *Pharus legumen*, transverse section through inhalant siphon. ×90. C_1–C_4, circular layers of collagen fibres; *H*, haemocoel; *IE*, epithelium lining cavity of siphon; L_1–L_3, layers of longitudinal muscle; *N*, nerve (one of eight); *OE*, epithelium of outer surface of siphon; *R*, radial muscle.

Ligament. This requires little comment; it was not considered necessary to examine or describe it in detail. It is opisthodetic, elongate and external (Fig. 1). There is a long inner ligament layer secreted by the mantle isthmus where the epithelial cells are notably lengthened. Posterior to this is a broader but narrow region where the outer ligament layer is formed (anterior secretion of this layer is necessarily slight) and behind this again a broad fusion layer. This extends back under the level of the calcareous substance of the valves with which it becomes incorporated. These three major regions are indicated in Fig. 2. Conditions are thus essentially similar to those described in detail (although with superseded terminology) for *Tellina tenuis* by Trueman (1949) and more generally for *Scrobicularia plana* and *Abra alba* (Trueman, 1953). The only differences in *Pharus* are the greater length of the ligament and its greater secondary attachment, by way of the fusion layer posteriorly, to the shell valves.

ORGANS IN THE MANTLE CAVITY

Foot and pedal muscles. The size of the foot has already been noted, even when fully contracted it is some half the length of the shell. Although obviously resembling that of the Solenidae in general form, to some extent it differs slightly from that organ and resembles the foot of the Tellinacea in the presence of a more defined and rather frilled lateral margin. In action it tends to open out rather than to grip the substratum by bulbous dilation as in *Ensis* or *Solen.* It is pink in colour, *Pharus legumen* being one of the few bivalves to possess haemoglobin. In young specimens there is a well-developed byssus gland.

Graham (1934*b*) has described the pedal musculature in a variety of species of the Tellinacea and in *Cultellus* (Solenacea) and his findings have been helpful in this work. In *P. legumen*, as shown in Fig. 2, there are anterior and posterior retractors and an anterior protractor. The last-named is inserted into the valves on the anterior side of the ventrally extending rib and dorsal to the posterior end of the insertion of the anterior adductor. The anterior retractors have one area of insertion, posterior to the strengthening shell rib, although consisting of two muscles which diverge almost immediately. The posterior retractors are inserted, as normally, into a triangular area immediately anterior to the insertion of the posterior adductor.

The anterior retractor has a single insertion as it does in the Tellinacea but this cannot be considered of systematic value because although there is usually a double insertion of this muscle in the Solenacae, in *Solen*, as recently shown by Owen (1959) it is single. However, conditions in *Pharus* do certainly resemble those in the Tellinacea the only difference being that the muscle usually divides further from the point of insertion than it does in *P. legumen.* In this species also the anterior retractors are the most internal of the extrinsic muscles of the foot whereas the opposite is the case in *Cultellus* (Graham, 1934*b*). But, despite earlier views to the contrary, this also has no systematic value, conditions varying greatly within the Solenacea as shown by Owen (1959).

Palps and Ctenidia. The palps are large, there being no such long proximal oral groove between them and the mouth as there is in Solenacea such as *Ensis siliqua* (Graham, 1934) or *E. arcuatus* (Yonge, 1952). They are much-ridged on the opposed faces and are clearly most efficient organs of selection.

With reference to the ctenidia, Ridewood (1903) states that, '*Ceratisolen* (i.e. *Pharus*) *legumen* has a non-plicate, homorhabdic gill, in striking contrast with those of other genera of the Solenidae'. However, the ctenidia of

Cultellus, undoubtedly a member of that family, are homorhabdic (Atkins, 1936) while in the Tellinacea, the ctenidia are homorhabdic in *Tellina*, *Macoma*, *Scrobicularia*, *Abra* and in *Donax vittatus* (Atkins, 1937*a*), although Ridewood (1903) found a variety of conditions in different species of *Donax*, some being homorhabdic and others heterorhabdic. In all species of *Gari* and *Solecurtus* examined by Ridewood (1903) and Atkins (1937*a*), the ctenidia were plicate, i.e. heterorhabdic.

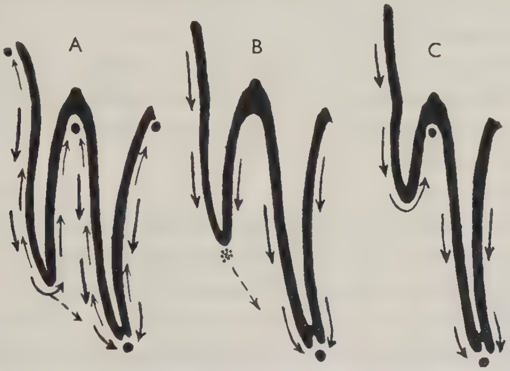


Fig. 7. Diagrams showing arrangement of ciliary currents on ctenidia of A, *Cultellus pellucidus*; B, *Pharus legumen*; C, *Donax vittatus* or *Gari* spp. (A and C after Yonge, 1949.)

A further point is the well-marked supra-axial extension of the outer lamellae of the outer demibranch. This is shown in Fig. 7B and the same condition is found very generally throughout the Tellinacea (Yonge, 1949, Fig. 22) where indeed it is much further developed in the Tellinidae (e.g. *Tellina*, *Macoma*) and Semelidae (e.g. *Scrobicularia*, *Abra*). Conditions in *Donax*, *Gari* (Fig. 7C) or *Solecurtus* most resemble those in *Pharus*. However, a slight supra-axial extension does also occur in *Cultellus* (Fig. 7A) and is well developed in a wide variety of other Eulamellibranchia. There remains for final consideration the ciliation. Here a real difference exists. In all Solenidae studied, i.e. *Ensis*, *Solen*, *Cultellus* (Atkins, 1936, 1937*a*) and *Siliqua* (Yonge, 1952), the ordinary filaments (in *Cultellus*, which is homorhabdic this means all filaments) bear both large and small frontal cilia (Fig. 7A). The former beat towards the free margins of the demibranchs and the latter in the opposite direction, i.e. towards the axis or the outer extremities of the demibranchs. The effect is to select the finer particles for conveyance oralwards along these three dorsal channels while the larger particles are conveyed to the marginal (so-called 'food') groove on the inner demibranch from which the greater part will fall off on to the mantle surface and be removed with the pseudofaeces.

In *Pharus legumen* (Fig. 7B) there are *no* small frontal cilia, all cilia beating marginally. There is a resemblance to the foregoing conditions in the presence of a marginal groove on the inner demibranch only but this is also true of all

Tellinacea (e.g. *Donax* or *Gari* as shown in Fig. 7C). The margin of the outer demibranch, however, bears a series of coarse marginal cilia precisely as described and figured in that region in *Gari fervensis* by Atkins (1937*b*, fig. 8). Also significant is the absence in *Pharus legumen* of any dorsal oralward current, conditions here differing from those in the Solenacea with three such dorsal currents (see Fig. 7A) and resembling those in the Tellinacea where in some species there are no such currents while in others, including species of *Donax* and *Gari* (Fig. 7C), there is one such current, along the ctenidial axis (see Yonge, 1949 for full details).

There is little doubt that on balance the ctenidia of *Pharus legumen* do reveal closer affinity to those of the Tellinacea, the absence of the double series of frontal cilia beating in opposite directions being a notable point of difference from the Solenidae.

Disposal of Waste. As indicated by the disposition of the feathered arrows in Fig. 2, waste material from all regions of the inhalant chamber, from the tips of the palps and from the ctenidia, is carried posteriorly along a ventral channel bounded by low ridges which arise in the region below the tips of the palps and extend to the base of the inhalant siphon. The general surface of this region and especially the crests of the ridges is richly ciliated (Fig. 3). Pseudofaeces collect at the base of the siphon for periodic expulsion. Much deeper ridges, better termed membranes, occur in *Siliqua patula* (Yonge, 1952) amongst the Solenacea and also in many Tellinacea (Yonge, 1949), although not in *Donax*, *Gari* or *Solecurtus*. Certainly no systematic value can be placed on this structural feature of obvious functional convenience.

ALIMENTARY CANAL

The general course of the gut in *Pharus legumen* is shown in dotted outline in Fig. 2. There is an unusually long oesophagus, the stomach is extended somewhat dorso-ventrally, the mid-gut is separated from the long style-sac, extending alongside and then for some distance beyond this well forward into the foot. It is thrown terminally into a series of tight coils (*MG*). It returns to pass dorsally to the right of the stomach and so, through the ventricle, to open at the anus behind the posterior adductor. The stomach easily dissects out in fresh material: it has a well-developed muscular wall with the contents, including sizable sand grains, in some degree of tension. There is a small postero-dorsal caecum (*PDC*), much as in *Solecurtus* (Yonge, 1949). The style is extremely stout and bears against a conspicuous gastric shield with a conspicuous tooth.

Significant matters are the character of the stomach and the deep penetration into the foot by the style-sac (*SS*) and mid-gut (*MG*). The stomach in the Tellinacea is highly characteristic having, in association with the deposit feeding habit, acquired many of the characteristics of a gizzard. These include an unusually stout style, very extensive gastric shield, muscular walls and a conspicuous postero-dorsal caecum peculiar to the group and apparently serving as a safety valve wherein excess particles are temporarily stored prior to trituration between style and gastric shield (Yonge, 1949). All of these features are present in *Pharus*, though not to so marked an extent as in genera such as *Tellina* and *Scrobicularia*. There is no resemblance to the stomach of the suspension feeding Solenacea. Although in *Pharus*, as in the Solenacea, style-sac and mid-gut are separate whereas they are united in the majority of the Tellinacea, this is not a feature of any phylogenetic significance and indeed the two are separate in *Donax* which certainly belongs to the Tellinacea. Deep penetration of the foot by style-sac and mid-gut does not occur in the Solenacea (Graham, 1931, 1934*b*; Owen, 1959).

SYSTEMATIC POSITION

Confining this discussion to essentials, of the various features discussed in this account of *P. legumen* the following may be regarded, from the standpoint of its affinities, as crucial:

Siphons. Their complete separation and formation exclusively from the inner folds of the mantle margin being ringed (inhalant siphon only) with a single row of simple tentacles; their complex internal structure of the type highly characteristic of the Tellinacea.

Ctenidia. Supra-axial extension but much more significant the absence of any dorsal oralward current and especially lack of the small frontal cilia and so of the mode of selection of particles on the gill surface so characteristic of the Solenacea.

Gut. General form of the stomach, especially the presence of a small postero-dorsal caecum found only in the Tellinacea; deep penetration of the mid-gut and style-sac into the foot, unlike the Solenacea.

No other character mentioned is entirely confined to the one group or the other but the above list provides sure grounds for the removal of *Pharus* from the Solenacea and its inclusion in the Tellinacea.

The major difference from the more obviously typical members of the Tellinacea is the absence of a cruciform muscle (Graham, 1934*a*; Yonge, 1949). But this is also true of *Novaculina* and *Sinovacula* (subfamily Novaculininae) which, it has previously been suggested (Yonge, 1949), should be removed from the family Solenidae where they were placed by Ghosh (1920) and transferred to the Tellinacea. This was on the basis of the formation of the siphons, unquestionably a crucial character because one not liable to adaptive modification. There is much greater supporting evidence for this transfer in the case of *Pharus* where the internal structure of the siphons is also known and highly significant differences in the ctenidia and gut also observed. The absence in these three genera of the cruciform muscle, otherwise apparently universally present in the Tellinacea, is very probably associated with the extension of pallial fusion (Type B in *Pharus* but of unknown type in the Novaculininae), along a greater part of the ventral surface; in the great majority of the Tellinacea fusion is confined to a very small region at the base of the siphons where the cruciform muscle acts as a tie for the elongate siphons (Yonge, 1949).

Elongation of the shell in a manner superficially very similar to what occurs in the Solenacea seems to have occurred several times in the Tellinacea. It has led to the appearance of *Tagelus* (with no ventral mantle fusion but with a cruciform muscle), of *Solecurtus* (with ventral mantle fusion but involving *only the inner mantle folds* i.e. Type A, and with a cruciform muscle) and of *Pharus*- and also probably of the separate group of the Novaculininae- (with more intimate ventral fusion and without a cruciform muscle). The major external difference of these elongate genera from the Solenacea is the approximately central position of the hinge and ligament, the mantle/shell having extended both anteriorly and posteriorly and not solely posteriorly. In the case of *Tagelus* the habits are also somewhat different, species of this genus occupy semi-permanent vertical burrows in mud. Species of *Solecurtus* live in deeper water and probably do not burrow deeply or perhaps even very quickly. The habits of the Novaculininae are uncertain but in *Pharus* they do resemble those of the Solenacea, living in shallow water and burrowing vertically both deeply and with great speed. An important difference may be their need, as deposit feeders, for silty conditions not present in the exposed habitat of most members of the Solenacea. In European waters *Solen* tends to occupy the environment favoured by *Tagelus* elsewhere.

SUMMARY

Pharus legumen (L.), a little-known member of the British marine fauna, is confined to the south-west and to the coasts of Wales. It inhabits a restricted zone on the margin of the lower shore and the shallow sublittoral where some silt is present.

Both in the elongation of the shell and in the habit of vertical burrowing, *P. legumen* resembles members of the Solenacea, i.e. species of *Ensis* and *Solen*.

Following description of this species with an account of the pallial structures, organs in the mantle cavity, nature of the ciliary currents on the mantle and the ctenidia, and the course of the alimentary canal, reason is given for the transference of *Pharus legumen* to the Tellinacea.

Major evidence for this decision comes from the separation of the siphons which are composed exclusively of the inner folds of the mantle margin and have the highly characteristic internal structure of those of the Tellinacea, the absence on the ctenidia of dorsal oralward currents and of the small frontal

cilia, both characteristic of the Solenacea, and finally from the nature of the stomach with its small postero-dorsal caecum and the deep penetration into the foot of the mid-gut.

The major difference from the Tellinacea generally is the absence of a cruciform muscle but this may be associated with the extensive ventral fusion of the mantle margins, not found in genera with a cruciform muscle with the exception of *Solecurtus* where it is less extensive and not so intimate (of Type A instead of Type B) as it is in *Pharus legumen.*

REFERENCES

ATKINS, D., 1936. On the ciliary mechanisms and interrelationships of lamellibranchs. Part I. *Quart. J. micr. Sci.*, Vol. 79, pp. 181–308.

—— 1937*a*. On the ciliary mechanisms and interrelationships of lamellibranchs. Part II. *Quart. J. micr. Sci.*, Vol. 79, pp. 339–73.

—— 1937*b*. On the ciliary mechanisms and interrelationships of lamillibranchs. Part III. *Quart. J. micr. Sci.*, Vol. 79, pp. 375–421.

BLOOMER, H. H., 1903. Classification of the British species of the genus *Solen. J. Malacol.*, Vol. 10, pp. 41–3.

CHAPMAN, G. & NEWELL, G. E., 1956. The rôle of the body fluid in the movement of soft-bodied invertebrates. II. The extension of the siphons of *Mya arenaria* L. and *Scrobicularia plana* (da Costa). *Proc. Roy. Soc.*, B, Vol. 145, pp. 564–80.

DESHAYES, G. H. 1845–48. Histoire naturelle des Mollusques. 1. Mollusques acéphalés. *Exploration scientifique de l'Algérie*, 1840–42. Sciences Physiques, Zoologie. Paris.

FORBES, E. & HANLEY, S., 1953. *A History of British Mollusca*, Vol. 1. London: Van Voorst.

FRAENKEL, G., 1927. Die Grabbewegung der Soleniden. *Z. vergl. Physiol.*, Bd. 6, pp. 167–220.

GHOSH, E., 1920. Taxonomic studies on the soft parts of the Solenidae. *Rec. Indian Mus.*, Vol. 19, pp. 47–78.

GRAHAM, A., 1931. On the morphology, feeding mechanisms, and digestion of *Ensis siliqua* (Schumacher). *Trans. roy. Soc. Edinb.*, Vol. 56, pp. 725–51.

—— 1934*a*. The cruciform muscle of lamellibranchs. *Proc. roy. Soc. Edinb.*, Vol. 54, pp. 17–30.

—— 1934*b*. The structure and relationships of lamellibranchs possessing a cruciform muscle. *Proc. roy. Soc. Edinb.*, Vol. 54, pp. 158–87.

HOFFMANN, F., 1914. Beiträge zur Anatomie und Histologie von *Tagelus dombeyi* (Lamarck). *Jena Z. Naturw.*, Vol. 52, pp. 521–61.

JEFFREYS, G., 1865. *British Conchology*, Vol. 3. London: Van Voorst.

OWEN, G., 1958. Shell form, pallial attachment and the ligament in the Bivalvia. *Proc. zool. Soc. Lond.*, Vol. 131, pp. 637–648.

—— 1959. Observations on the Solenacea with reasons for excluding the family Glaucomyidae. *Phil. Trans.* B, Vol. 242, pp. 59–97.

RAWITZ, B., 1892. Der Mantelrand der Acephalen. Dritter Teil. *Jena Z. Naturw.*, Bd. 27, pp. 1–232.

RIDEWOOD, W. G., 1903. On the structure of the gills of the Lamellibranchia. *Phil. Trans.* B, Vol. 195, pp. 147–284.

ROSCOFF, 1951. Inventaire de la Faune Marine de Roscoff. Mollusques. *Trav. Sta. biol. Roscoff*, Suppl. 5.

THIELE, J., 1935. *Handbuch der systematischen Weichtierkunde.* Dritter Teil. Classis Bivalvia. Jena: G. Fischer.

TRUEMAN, E. R., 1949. The ligament of *Tellina tenuis*. *Proc. zool. Soc. Lond.*, Vol. 119, pp. 719–42.

—— 1953. The structure of the ligament of the Semelidae. *Proc. malacol. Soc. Lond.*, Vol. 30, pp. 30–6.

YONGE, C. M. 1949. On the structure and adaptations of the Tellinacea, deposit-feeding Eulamellibranchia. *Phil. Trans.* B, Vol. 234, pp. 29–76.

—— 1952. Studies on Pacific coast Mollusks. IV. Observations on *Siliqua patula* and on evolution within the Solenidae. *Univ. Calif. Publ. Zoöl.*, Vol. 55, pp. 421–38.

—— 1957. Mantle fusion in the Lamellibranchia. *Pubbl. staz. zool. Napoli*, Vol. 29, pp. 151–71.

J. mar. biol. Ass. U.K. (1959) 38, 291–312 (*N.I.R.D. Paper No.* 2155)
Printed in Great Britain

THE FOOD OF *MEGANYCTIPHANES NORVEGICA* (M. SARS), WITH AN ASSESSMENT OF THE CONTRIBUTIONS OF ITS COMPONENTS TO THE VITAMIN A RESERVES OF THE ANIMAL

By L. R. FISHER and E. H. GOLDIE

Unit for Biochemical Research bearing on Fisheries' Problems[1] at the National Institute for Research in Dairying, Shinfield, Reading

(Text-figs. 1–10)

The importance of *Meganyctiphanes norvegica* (M. Sars) as a source of vitamin A for its predators has been demonstrated by Fisher, Kon & Thompson (1955). Basing their conclusions on the observations of Macdonald (1927) on the feeding of *M. norvegica* in Loch Fyne, Fisher, Kon & Thompson (1954) and Kon (1954) suggested a possible scheme of accumulation of vitamin A in the animal. They also made cursory examinations of the stomach contents from animals caught in the same area and their findings appeared to agree fairly well with those of Macdonald, except that from his results he concluded that phytoplankton was a major item in the diet. This conclusion has been disputed by Einarsson (1945) who called for more detailed investigation of fresh material in order to obtain a clearer picture of the feeding of north Atlantic euphausiids. Such information would be of great value in the study of the vitamin A chain in the plankton. We have, therefore, examined the stomach contents of *M. norvegica* over nearly two years to try to detect every type of food that might be eaten. In addition to our own survey, Dr J. Mauchline (1959) of the Marine Station, Millport, made a more intensive study of the food in relation to the diurnal migrations in July and November.

Earlier work on the food of *M. norvegica* in different areas records the species feeding on copepods, schizopod and decapod larvae, diatoms, dinoflagellates and detritus (Holt & Tattersall, 1905; Paulsen, 1909; Hickling, 1923–5). Macdonald (1927) concluded that larger specimens feed mainly on copepods, small specimens on diatoms and 'wet dust' or flocculent detritus, and intermediate sizes on vegetable detritus. Einarsson (1945) regarded the chief items in the diet to be detritus and crustacean fragments. He also mentioned the occurrence of a reddish-brown substance in the anterior part of the stomach. The nature of this substance has been suggested by Ponomareva (1955), who found in the stomachs of four species of Pacific euphausiids the

---

[1] Grant-aided by the Development Fund.

crystalline cones of compound eyes and also remains of the eyes themselves; she considered the dark reddish-brown mass to be the pigmented part of the eyes. The pigment rapidly disappeared on passage through the gut. Ponomareva examined different size-groups from the cyrtopia to the adult stages and found that the groups differed little in the composition of their food; even small euphausiids fed on copepods. She agreed with Einarsson that phytoplankton is not a basic food of euphausiids.

The local environment might account for the presence of vegetable tissues from terrigenous deposits in the Loch Fyne specimens of *M. norvegica* (Macdonald, 1927) and different material might be found in those living farther offshore. In stomachs of *Euphausia krohnii*, caught in the Bay of Biscay, we found contents similar to those of *Meganyctiphanes norvegica*, but none of the terrestrial material present in the stomachs of *M. norvegica* from Loch Fyne. Nevertheless, although *M. norvegica* may be considered as living under specialized conditions in this loch, such sea areas with deep water in close proximity to the land may be important fishing grounds. *M. norvegica* is often a component of the plankton in such areas, not only in Loch Fyne but also in, for example, the Skagerak (Poulsen, 1926), the fjords of the coast of Möre (Ruud, 1926) or the Gulf of Maine (Bigelow, 1914).

MATERIAL AND METHODS

Samples of *Meganyctiphanes norvegica* were collected with 1 m or 2 m stramin nets on one or two consecutive days during each month from September 1956, to April 1958, and in June 1958. The samples for November and December 1957 were kindly collected for us by Dr Mauchline. The area fished in Loch Fyne extended from just north of Arran in a north-westerly direction to the deep water north-east of Tarbert. Apart from one haul taken at about midnight in October 1956, all the specimens examined in 1956 and 1957 were caught during the hours of daylight between 8 a.m. and 6 p.m. A haul was taken at midnight in each monthly series during the period January to April 1958, and in June 1958.

The animals were size-grouped from the length of the carapace at 1 mm. intervals to the nearest half millimetre. Mauchline (1959) discusses the relationship of this measurement to those used by earlier workers, including ourselves. The sexes were not examined separately. Size relationships of the specimens studied are given in Table 1.

Detritus, plant material and copepods were often found in the 'basket' formed by the thoracic legs, but it seems likely that at least some of these, especially whole copepods, such as *Pareuchaeta norvegica*, became adventitiously entangled when the animals were in the net, and so we have assumed that only material that had actually entered the stomach should be considered as food.

In collaboration with Dr Mauchline, we took, with a Jenkin's mud sampler (Mortimer, 1942), kindly lent by the Freshwater Biological Association, mud samples from Loch Fyne, in the area where most of the *Meganyctiphanes norvegica* were caught. The cores, of about 3 in. diameter, were taken on 7 March 1957, at a depth of 175 m. They were cut into sections about 2½ in. long, except the surface layer which was about ½ in. thick and included the thin layer of water lying above it. All the cores were preserved by shaking with about twice their volume of absolute alcohol. Fat-soluble material extracted from the mud was analysed by our usual method (Fisher, Kon & Thompson, 1952).

TABLE 1. SIZE DISTRIBUTION OF SPECIMENS OF *MEGANYCTIPHANES NORVEGICA* EXAMINED

Date of hauls	Time	Number of specimens of carapace length (mm)											Total
		2	3	4	5	6	7	8	9	10	11	12	
19–20. ix. 56	D	28	22	24	17	2	0	11	22	6	9	1	142
25–26. x. 56	D	2	6	28	89	35	21	14	13	12	9	1	230
25–26. x. 56	N	0	0	0	1	14	10	0	2	3	0	1	31
22–23. xi. 56	D	0	1	9	37	21	1	35	19	4	0	0	127
19–20. xii. 56	D	0	0	4	28	8	0	32	18	6	0	0	96
9–10. i. 57	D	0	0	3	40	24	3	51	8	2	0	0	131
7–8. ii. 57	D	0	0	2	27	12	3	20	3	0	0	0	67
7–8. iii. 57	D	0	0	7	28	5	1	9	1	0	0	0	51
4–5. iv. 57	D	0	0	0	10	19	7	37	14	3	0	0	90
24. iv. 57	D	0	0	0	0	6	5	7	9	1	0	0	28
22–23. v. 57	D	0	0	0	1	6	34	27	2	2	0	0	72
19–20. vi. 57	D	0	1	1	0	6	65	48	6	1	0	0	128
10–11. vii. 57	D	0	3	42	41	3	15	15	0	0	0	0	119
14–15. viii. 57	D	0	0	2	48	86	38	116	3	2	0	0	295
11–12. ix. 57	D	0	0	0	4	16	36	97	11	0	0	0	164
9–10. x. 57	D	0	0	1	3	12	64	176	26	0	0	0	282
19. xi. 57	D	0	0	0	1	3	45	72	5	0	0	0	126
17. xii. 57	D	0	0	0	0	1	31	81	2	0	0	0	115
15–16. i. 58	D	0	0	0	0	4	42	150	9	0	0	0	205
15–16. i. 58	N	0	0	0	0	2	27	80	5	0	0	0	114
19–20. ii. 58	D	0	0	0	0	10	57	118	2	0	0	0	187
19–20. ii. 58	N	0	0	0	0	7	41	119	3	1	0	0	171
27–28. iii. 58	D	0	0	0	4	9	58	128	2	0	0	0	201
27–28. iii. 58	N	0	0	0	0	3	21	87	1	0	0	0	112
15–16. iv. 58	D	0	0	0	17	13	51	94	2	0	0	0	177
15–16. iv. 58	N	0	0	0	5	7	57	102	1	0	0	0	172
25–26. vi. 58	D	0	0	0	0	0	10	62	10	0	0	0	82
25–26. vi. 58	N	0	0	0	0	0	0	36	13	1	0	0	50
Total		30	33	123	401	334	743	1824	212	44	18	3	3765

D = Caught in daylight. N = Caught at night.

RESULTS

In considering intensity of feeding and types of food eaten we have first combined results for animals of all sizes in each monthly group. We then discuss the possible relationship between size and the kind of food eaten. So that the results should be comparable throughout the period of sampling, only day hauls are considered. Finally, the results for night and day hauls are compared.

Intensity of feeding

The intensity of feeding was determined by calculating the percentage of animals with food in the stomach in each monthly haul. However, some stomachs contained only a little detritus or other food, contrasting with others from animals that had obviously been feeding more intensively. From November 1956, a note was, therefore, made of the degree of fullness of the stomach.

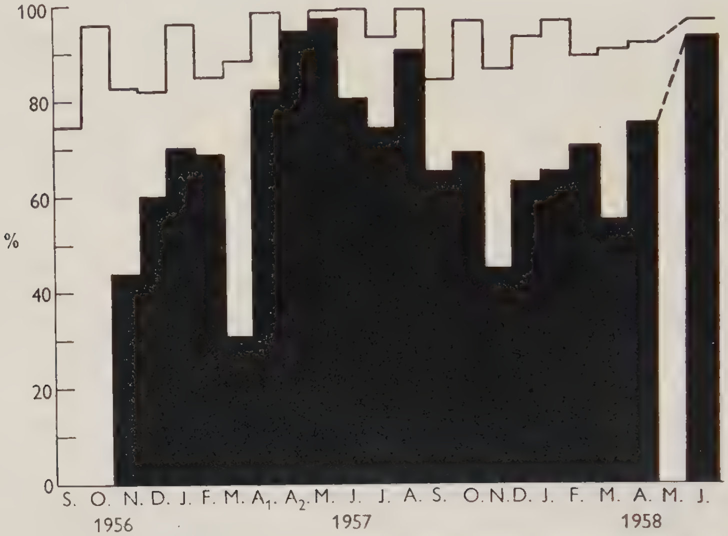


Fig. 1. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing food. Shaded portion: percentage with stomachs more than half-full.

Feeding intensity reached its peak during the spring plankton outburst in late April and May (Fig. 1). The high intensity of feeding in August was associated with the presence of numerous young euphausiids and dinoflagellates in the plankton. There was also evidence of a smaller increase corresponding to the autumn plankton surge in October 1956 and 1957. The high percentage of animals with nearly full stomachs in January and February of each year was unexpected. In January 1957, it was at first thought to be associated with an abundance of the copepod *Pareuchaeta norvegica*. In 1958, however, the species was not particularly numerous in these months. We now consider it more likely that increased feeding at this time of year is due to the greater activity associated with spermatophore transference (Mauchline, 1959).

Types of food

The main components of the diet of *Meganyctiphanes norvegica* from Loch Fyne were organic debris, mud particles and other inorganic detritus, Crustacea, dinoflagellates, fern sporangia, dipteran egg membranes, diatoms, algae, and occasionally other items of terrestrial or marine origin.

Organic debris and inorganic detritus were present in many stomachs, as would be expected during daylight when these diurnally migrating animals lie on or near the bottom. Fig. 2 shows the seasonal fluctuations of these two components. The large amounts usually found indicated that this material probably forms the bulk of the diet, except possibly during the spring feeding period.

There was a marked seasonal variation in the occurrence of crustacean fragments in the stomachs (Fig. 3 A). Owing to lack of time no attempt was made to identify parts of copepods separately, and all crustacean remains are considered together, save for the easily identified compound eye parts. The presence of these eyes indicates feeding on eucaridan Crustacea and their absence, when the incidence of crustacean fragments remains high, indicates that copepods were the Crustacea eaten, as during the spring increase in April and May.

In Fig. 3 B the percentage of stomachs containing crustacean remains including compound eye parts is shown, and the two periods, June–November 1957 and January–April 1958, are those with the highest frequencies. From June onwards late larvae and adolescents of euphausiids are numerous in the plankton and many of the eyes in the stomachs were identified as belonging to euphausiids. The higher incidence of crustacean fragments and eyes in the early months of 1958 than in the same period in 1957 is difficult to explain, but may have been associated with the absence of sufficient alternative food in the water. It is possible that cannibalism occurs when the proportion of euphausiids to other organisms in the plankton is high either because of an abundance of euphausiids, as in the late summer, or because of a scarcity of other plankton organisms, as in the winter. On the other hand, the average size of the *M. norvegica* forming the population sampled was higher in January 1958 (carapace 8·0 mm long) than a year earlier (6·7 mm) and larger animals eat more crustaceans (see p. 299). However, in the smallest size-group (6 mm carapace) for which we have adequate values for both years there were many more animals with stomachs containing crustacean fragments in January 1958 (75%) than in January 1957 (46%), which suggests that the composition of the plankton is the determining factor.

Dinoflagellates were most frequently found in late summer and autumn with minor increases in April and January (Fig. 4). When dinoflagellates were abundant in the plankton they formed an important part of the diet of *M. norvegica*, occurring in the stomachs in enormous numbers. The species most frequently found belonged to the genera *Ceratium*, *Dinophysis*, *Phalacroma*, *Prorocentrum* and *Peridinium* (Fig. 5). *Ceratium* occurred commonly in autumn, 1956, but in the corresponding period in 1957 it was replaced by *Dinophysis* and *Prorocentrum*. A few stomachs contained *Goniaulax*, mostly in

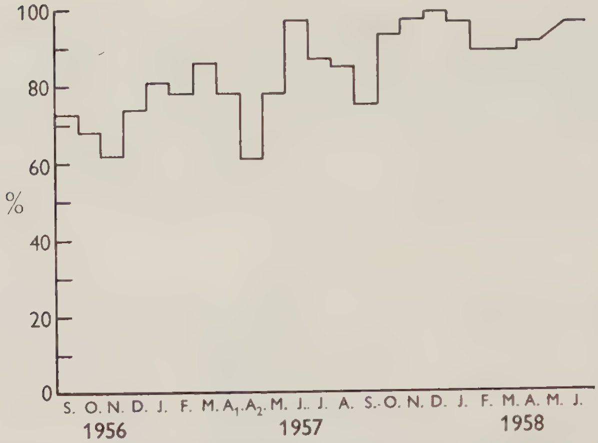


Fig. 2. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing organic debris and inorganic detritus.

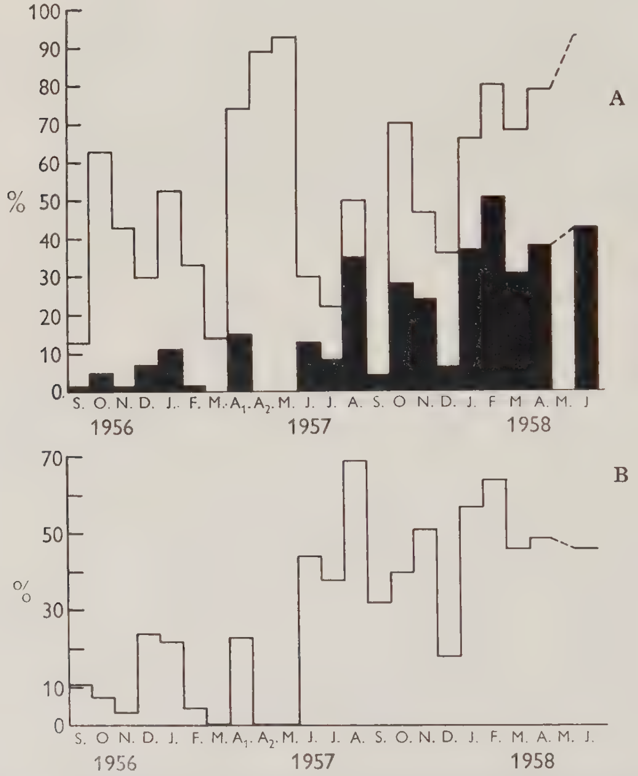


Fig. 3. A, Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing crustacean fragments. Shaded portion: percentage with stomachs containing compound eyes; B, Seasonal variation in percentage of those *M. norvegica* with stomachs containing crustacean fragments in which compound eyes were also present.

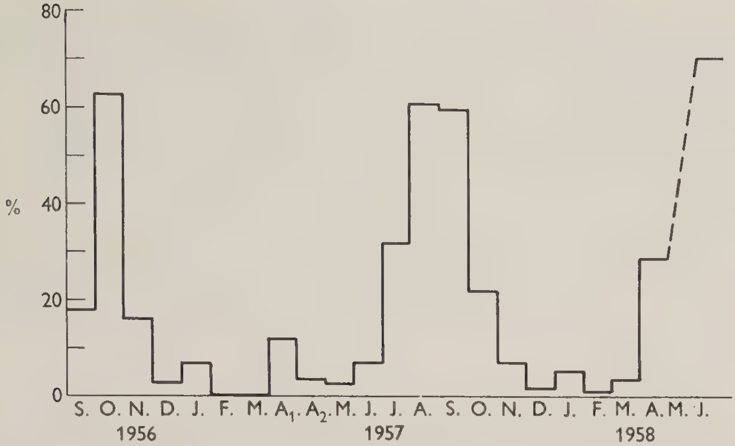


Fig. 4. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing dinoflagellates.

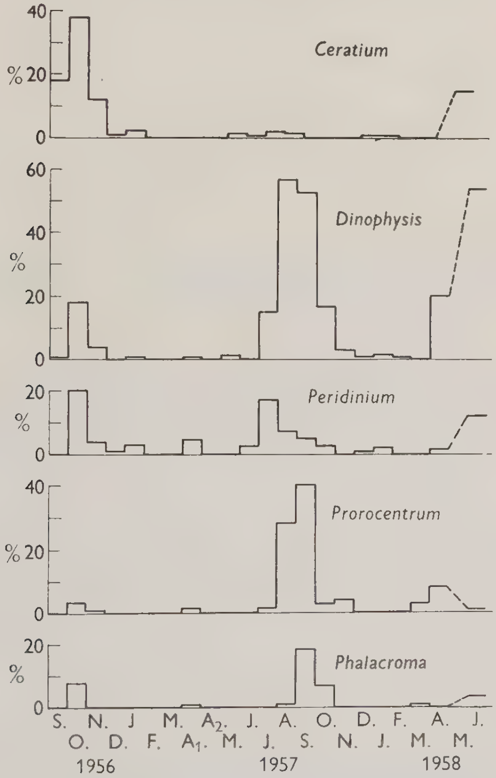


Fig. 5. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing different genera of dinoflagellates.

the autumn. *Noctiluca* appeared in small numbers intermittently throughout the year, and the only other genus found was *Peridiniopsis* in two stomachs in August 1957.

Fern sporangia appeared in the stomachs first in the late spring and continued through the summer, reaching their maximum in the autumn (October 1956, and August 1957) (Fig. 6A).

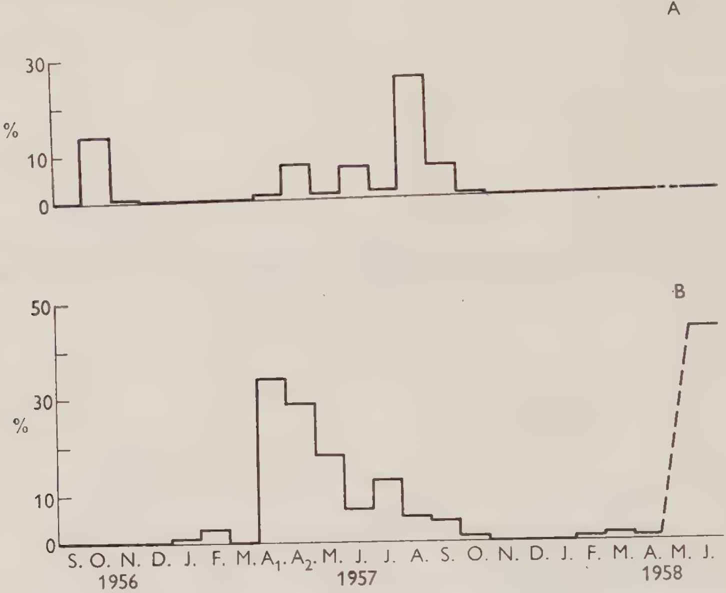


Fig. 6. A, Seasonal variation in percentage of stomachs of *Meganyctiphanes norvegica* containing fern sporangia. B, Seasonal variation in percentage of *M. norvegica* with stomachs containing dipteran egg membranes.

Structures tentatively identified as the chorionic membranes of the eggs of a dipterous insect, probably a chironomid or ceratopogonid, were found in the stomach contents from April to October in 1957, with maximal incidence at the beginning of the period (Fig. 6B). Some species of these insects are present in large numbers on the west coast of Scotland. An unsuccessful attempt was made to identify the membranes by comparison with eggs taken from female chironomids of different species, kindly provided by Dr N. C. Morgan of the Brown Trout Research Laboratory, Pitlochry, although there was a close resemblance to some of these eggs. The seasonal occurrence of the membranes in the stomachs accorded well with that of the breeding season of chironomids in the vicinity (Stuart, 1945).

Seasonal variations in the numbers of stomachs with diatoms (Fig. 7) corresponded to the annual fluctuations of these organisms in the plankton. In the first months of 1958 the numbers of stomachs with diatoms increased from January to April, more closely following the expected cycle of the plankton than in the previous year. Diatoms were never very numerous in the individual stomachs and were not at any time predominant in the contents. The most commonly occurring were species of *Paralia* and *Thalassiosira* and more rarely we found *Coscinodiscus*, *Rhizosolenia*, *Navicula* and *Biddulphia*.

Parts of green, red and brown algae appeared in a small proportion of stomachs throughout the year; the species were not determined. Fern sporangia, insect egg membranes, diatoms, algae and such occasionally occurring items in the stomachs as chrysomonads and radiolarians, sponge spicules, *Sagitta* spp., terrestrial plant material, and various unidentifiable structures were quantitatively unimportant components of the diet.

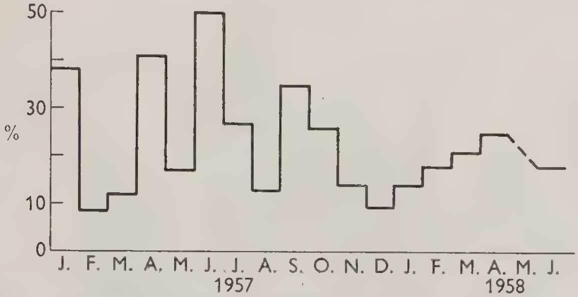


Fig. 7. Seasonal variation in percentage of *Meganyctiphanes norvegica* with stomachs containing diatoms.

Food in relation to size of animal

Stomachs were examined from animals of carapace lengths ranging from 2 to 12 mm. The smallest of these were probably late furcilia stages (Einarsson, 1945); otherwise, all the animals were post-larval. As specimens with carapace lengths of 2, 11 and 12 mm were taken only during September and October 1956, they have been disregarded partly because of their small numbers and partly because of the seasonal bias they would impart. There appeared to be no overall relationship between size and feeding intensity (Fig. 8A).

The percentage of animals with organic debris and inorganic detritus tended to increase with size (Fig. 8B). Since these components are probably mainly derived from the sea floor there is evidence that larger specimens spend a longer time there than smaller ones. By taking net hauls at different depths Mauchline (1959) has demonstrated the vertical layering of size-groups with the largest specimens lowest in the water.

The incidence of crustacean remains in the stomachs increased fairly sharply with size in the smaller animals, reaching a maximum in those of carapace length 8 mm. and then falling slightly (Fig. 8C). Our values were not so high and did not show so simple an increase to the largest size-group as those illustrated by Mauchline (1959), but his results were based on a single set of hauls whereas our histogram is compiled from all our values. The difference may be due to seasonal variation, but no definite indication of it could be derived from an analysis of the monthly values. Compound eyes in stomach contents increased in number with the size of the animals, reaching 28% in the group with carapace length 8 mm. and then falling rather sharply (Fig. 8D). These percentages are higher than those of Mauchline (1959), undoubtedly owing to the much higher values we recorded between October 1957, and June 1958.

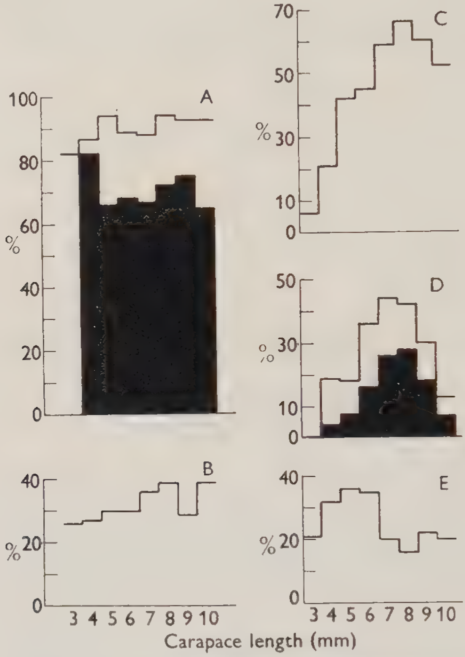


Fig. 8. A, Percentage of *Meganyctiphanes norvegica* of different size-groups with stomachs containing food. Shaded portion: percentage with stomachs more than half-full. B, Percentage of *M. norvegica* of different size groups with stomachs containing organic debris and inorganic detritus. C, Percentage of *M. norvegica* of different size-groups with stomachs containing crustacean fragments. D, Percentage of those *M. norvegica* of different size-groups with stomachs containing crustacean fragments in which compound eyes were also present. Shaded portion: percentage of all stomachs in each size-group with compound eyes present. E, Percentage of *M. norvegica* of different size-groups with stomachs containing dinoflagellates.

Dinoflagellates occurred in all size-groups but most frequently in those with carapace lengths from 4 to 6 mm (Fig. 8E). Diatoms were most frequently present (18–20%) in animals of carapace lengths 6–8 mm. Fern sporangia occurred in all size-groups, with the incidence slightly higher in those with carapace lengths from 4 to 6 mm (7–11%). There was no indication that any size of euphausiid was more likely to contain dipteran egg cases, which were found in from 2 to 10% of animals in the size range 3–9 mm inclusive. Algae were absent from the smallest and largest animals and occurred in 6–10% of those animals with carapaces 4–9 mm long.

Seasonal variation of food in relation to size

The intensity of feeding by animals of different size-groups was calculated. Sufficient values were available to construct histograms only for the groups with carapace lengths of 6, 7 and 8 mm. In the 8 mm group (Fig. 9), which contained fairly large numbers of specimens in each month, intensive feeding occurred from December 1956 to February 1957, from April to June 1957, and in August 1957. The winter feeding period occurred again from December 1957 to February 1958. Although there were some small differences, the overall picture presented by the 7 mm group was similar to that for the 8 mm specimens. Animals of this size did not appear in any numbers until January 1957, and feeding activity was at its highest in February–March, late April–June and August–October 1957. Increased spring feeding began again in April and continued through June 1958. Maximal feeding periods for the 6 mm group occurred in December 1956 to February 1957, April–June, August–October 1957, and December 1957 to January 1958. The picture presented by all three size-groups and the fragmentary information for the larger and smaller sizes leaves no doubt that there are three main feeding periods during the year, the most intensive in spring and early summer, and the other two in autumn and in winter.

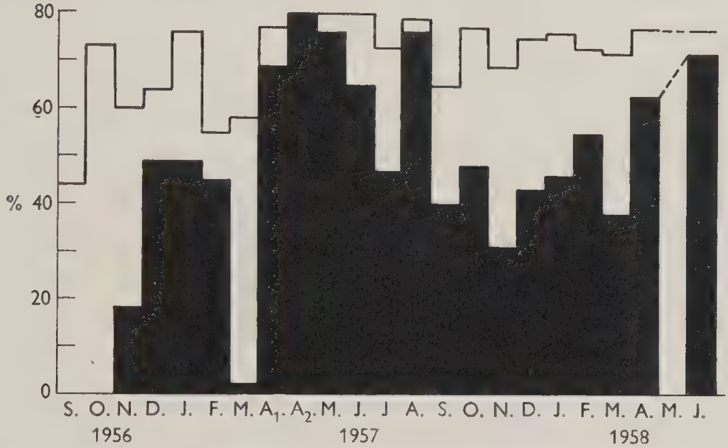


Fig. 9. Seasonal variation in percentage of *Meganyctiphanes norvegica* with a carapace length of 8 mm with stomachs containing food. Shaded portion: percentage of this size-group with stomachs more than half full.

As in the total population, seasonal incidence of organic debris and inorganic detritus in the various size-groups showed no consistent fluctuations.

The 8 mm group provided the most complete picture of the seasonal variations of crustacean remains in the stomachs and is representative of the other sizes. Fig. 10 A shows that the main peak was, as would be expected, in April and May 1957, although, in 1958, with no information for May, the highest level did not occur until June. January 1957 and February 1958 marked the peaks of the winter feeding periods for this kind of food. August and October were also months in which Crustacea were present in a high proportion of stomachs, with a marked contrast in September. In the 8 mm size-group there were no animals with such food in September 1956, and those of other sizes showed a low incidence in the same month. The close similarity between the fluctuations in Fig. 10 A and those in Fig. 3 A is remarkable since the 8 mm size-group did not constitute the majority of the population until September 1957.

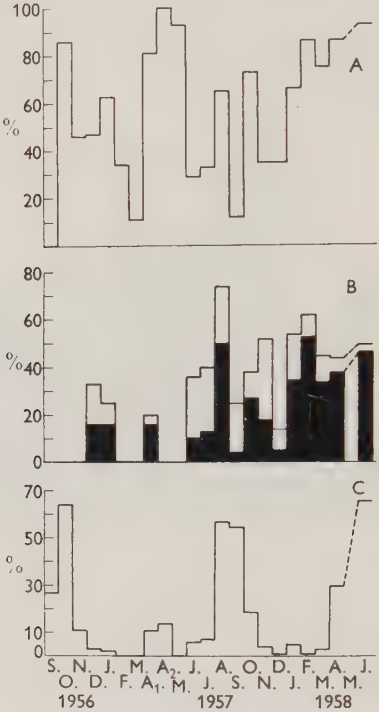


Fig. 10. A, Seasonal variation in percentage of *Meganyctiphanes norvegica* with a carapace length of 8 mm with stomachs containing crustacean fragments. B, Seasonal variation in percentage of those *M. norvegica* with a carapace length of 8 mm with stomachs containing crustacean fragments in which compound eyes were also present. Shaded portion: percentage of all stomachs in 8 mm group with compound eyes present. C, Seasonal variation in percentage of *M. norvegica* with a carapace length of 8 mm with stomachs containing dinoflagellates.

Our values for the occurrence of compound eyes in the stomachs of animals with 8 mm carapaces are again the most complete and are representative of the other sizes. Incidence of these eyes in the winter and autumn feeding periods was high but they were almost entirely absent from the stomachs of animals caught during the main feeding period in the spring (Fig. 10B). There are probably two reasons for this absence; one is that copepods are far more numerous during the spring increase than at other times and the other is that the euphausiids themselves exhibit much less luminescence at this time of year (Mauchline, 1959). Most of the eyes in the stomachs appear to be those of euphausiids, and their predators, whether of the same or another species, are probably attracted to them by the luminescing of the photophore on the eye-stalk.

Dinoflagellates in the stomachs exhibited the same seasonal trends in all size-groups. Fig. 10C shows these for the 8 mm group, with peaks in October 1956, and August and September 1957. In 1958, a peak as high as those in previous years appeared already in June when the investigation ended. The incidence of the different genera of dinoflagellates exhibited the same seasonal patterns in each size-group as shown by the whole population irrespective of size and illustrated in Fig. 5.

Diatoms, chorionic membranes of dipteran eggs and fern sporangia showed seasonal variations in the size groups similar to those in the whole population (see Figs. 6 and 7).

Diurnal variations in stomach contents

Table 2 shows that there was little difference in the occurrence of food in the stomachs of animals collected during daylight compared with those collected at around midnight, except in October 1956, when the number of stomachs with food was lower at night than in the daytime. This finding is, similar to that of Mauchline (1959) in November for *I*-group animals, that is, those that have taken part in one breeding season. The relationship between the intensity of feeding by day and by night in different size-groups was similar to that obtaining for the whole samples regardless of size.

The incidence of those animals with stomachs more than half full was higher at night in January, March, April and June, and lower in February. The degree of fullness of the stomach was not noted for the October specimens. The occurrence of organic debris and inorganic detritus was very similar in all the day and night hauls irrespective of size of animals examined. When feeding was intensive, especially in spring, the uptake of detritus was lower and no relationship between the size of the animals, their vertical distribution, and the incidence of such material was detected. During periods of less intensive feeding a marked correlation was apparent; with increasing size of animal and, therefore, a lower position in the water, more stomachs contained organic and inorganic particles.

As fern sporangia were present more frequently at night, when the animals normally migrate upwards, we infer that the sporangia occur in the plankton and not resting on the bottom. The incidence of dipteran egg cases did not vary so markedly between day and night, the most appreciable difference being in June when the daytime value was higher. However, since in that month feeding appeared to be at its peak, it seems likely that the egg membranes were in the plankton rather than on the bottom.

TABLE 2. INCIDENCE OF FOOD AND ITS COMPONENTS IN STOMACHS OF *MEGANYCTIPHANES NORVEGICA*

(Figures are percentages of all stomachs examined)

	Time caught	October 1956	January 1958	February 1958	March 1958	April 1958	June 1958
Food present in stomach	D	96	96	89	90	92	96
	N	81	97	85	93	94	98
Stomach more than half full	D	—	65	70	55	75	93
	N	—	70	49	92	92	98
Organic debris and inorganic detritus	D	68	96	89	89	91	96
	N	74	97	82	92	94	98
Crustacean fragments	D	63	66	79	68	79	93
	N	61	80	65	92	74	98
Compound eyes	D	4·8	37	51	31	38	43
	N	0	59	35	21	14	72
Fern sporangia	D	14	0	0	0	0	0
	N	39	0	0·6	1·8	0·6	2·0
Dipteran egg membranes	D	0	0	0·5	1·5	1·1	44
	N	0	0	1·8	1·8	1·1	34
Algal filaments	D	6·9	0·5	0·5	12	2·3	6·1
	N	32	0	0·6	5·4	5·8	4·0

D = Day. N = Night.

When dinoflagellates were abundant, as in October 1956 and June 1958, they were eaten by animals of all sizes by day and by night. In the first four months of 1958, when these organisms were found less often in the stomachs, the incidence of the three commonest genera, *Ceratium*, *Dinophysis* and *Peridinium*, tended to be higher during the day than at night and higher in smaller animals than in larger ones. *Prorocentrum* occurred in appreciable numbers in October and June and *Phalacroma* only in October and, on each occasion, they were more numerous in the animals caught at night. The difference in occurrence of these various genera by day and by night was probably associated with their vertical distribution in the plankton.

Diatoms were relatively unimportant in the diet. Usually *Thalassiosira* was found more frequently in the stomachs of specimens taken at night and *Paralia* in those taken in daylight. There was a tendency for diatoms to occur more often in the smaller animals in January, February and March, but in April and June, when they became more common in the plankton, they were found in about 20 to 30% of nearly all size-groups, with no predominance by day or night.

In October, algal filaments occurred during the day in size-groups up to and including 7 mm, and at night 9 mm, but the incidence was much higher at night than during the day. In the other months of the diurnal study algae occurred much less often and no relationship either to size or time of catching the animals was detectable.

Analysis of mud from bottom of Loch Fyne

Mauchline (1959) has given a brief account of the organisms found on the surface of the mud samples. Living filamentous algae were present, some of littoral origin, but none was attached or growing at that depth. There were also numerous ciliates. Like Mauchline, we found protozoan remains in the stomachs of the euphausiids. Only a few diatoms, mostly *Biddulphia*, were found in the mud. Small polychaetes were present in the deposits and were occasionally also found in the stomachs. The mud contained large numbers of cumaceans and a few isopods. If these were present in the stomachs they were included with the crustacean remains and not identified separately.

TABLE 3. CAROTENOIDS IN MUD FROM THE BOTTOM OF LOCH FYNE

Depth of samples = 175 m.

Sample	Thickness (in. approx.)	Total carotenoids		β-carotene	
		μg	μg/g fat	μg	μg/g fat
A. Surface	½	18	421	Trace	—
A. 2nd layer	2½	55	1070	4·5	87
A. 3rd layer	2½	116	6250	11	574
A. Bottom layer	2½	112	3380	13	397
B. Surface	½	22	3940	Trace	—
C. Surface	½	23	1050	Trace	—

Analysis of extracts from some of the cores for vitamin A and carotenoids (Table 3) shows that vitamin A was not present in measurable amount. The carotenoid content of the organisms just mentioned as living on the surface of the mud would be included in the values given in the table for the surface layers.

DISCUSSION

Although our findings provide new information about the food and feeding habits of *Meganyctiphanes norvegica*, confirming and supplementing that presented by Macdonald (1927) and Mauchline (1959), the picture is obviously very complex. We believe we now have enough information from this study about the qualitative aspects of the diet, although the proportions of its components have been derived from subjective estimates, to try to assess the contributions of the food to the rich reserves of vitamin A present mainly in the eyes. In addition we possess a considerable amount of data, from earlier observations of other workers and ourselves, on the occurrence of vitamin A or its precursors in these components.

The most commonly occurring food substances were inorganic detritus and organic debris, particularly in the stomachs of animals caught during the day when they were on or near the sea bottom and filter-feeding in the manner described by Mauchline (1959). Fox (1950) discussed the value of such material in the nutrition of marine animals and stressed its importance as a source of carotenoids. Marine muds have been shown to be particularly rich in carotenes (Fox, Updegraff & Novelli, 1944). Our own results (Table 3) indicate that, although β-carotene is present in the mud, a euphausiid that consumed the mud occupying a circular area of 3 in. diameter and $\frac{1}{2}$ in. thickness would not obtain enough to be measured by our technique (i.e. probably less than 1 μg). Certainly it would take in a greater amount of other carotenoids, but these would mostly be xanthophylls which, so far as we know, would not be vitamin A precursors. With allowance for greater thickness of the deeper mud samples there was some indication of an increase in carotenoid concentration and in the proportion of β-carotene with depth. Fox & Anderson (1941) found a similar increase in the ratio of carotenes to xanthophylls with the depth and, therefore, age of the mud. Our observations of living *Meganyctiphanes norvegica* in aquaria show that the animals do not penetrate far below the mud surface and so they would be unlikely to reach the layer richer in carotene.

Many stomachs contained what Mauchline (1959) has described as a 'green mush' of small particle size which he believes to be synonymous with Macdonald's (1927) 'flocculent detritus'. We do not know whether this material was derived from the bottom or from suspended matter in the sea water itself. Fox (1937) attempted to assess the importance of suspended matter and microplankton as a source of carotenoids by filtering 4000 l. of sea water. He extracted from the deposit 0·1 mg of xanthophylls and 0·02 mg of carotenes. Fox, Isaacs & Corcoran (1952) estimated that, in the sea off the Californian coast, the colloidal or otherwise finely particulate matter, which they called leptopel, included only 1·5–4% of living cells. Loch Fyne is less than 3 miles wide in the area where most of the euphausiids were caught, and the heavy rainfall (Barnes & Goodley, 1958), washes down a considerable quantity of detritus from the land, evidence of which has been provided by the occurrence of fern sporangia and dipteran egg cases in the stomachs of *Meganyctiphanes norvegica*. It is probable, therefore, that these waters contain more suspended material than those examined by Fox and his colleagues and are consequently a richer source of carotenoids than their data would suggest.

Crustacea become increasingly important in the food of *M. norvegica* as it grows. During the spring increase, copepods predominate but at other times of the year the presence of compound eyes indicated that eucaridan species are more commonly eaten. Many of these eyes were undoubtedly of euphausiid origin. In bulk of material the most important copepods in the planktonic community inhabited by *M. norvegica* are *Calanus finmarchicus* and *Pareuchaeta norvegica*. We have found occasionally small amounts of vitamin A in *P. norvegica* (Fisher *et al.* 1952) but none at all in *Calanus finmarchicus* (Fisher *et al.* 1952; cf. Euler, Hellström & Klussmann, 1934, and Lederer, 1938). We have now analysed twenty-two other species of copepod and found the vitamin in only two species of *Gaetanus* from the Bay of Biscay (Fisher & Kon, 1959). All these results refer only to the ester and alcohol forms of vitamin A. The recent finding of vitamin A aldehyde in fish eggs (Plack, Thompson & Kon, 1958) indicated that this form might be present in other marine organisms but not detected by our normal analytical technique. Using the gradient elution method of Plack, Kon & Thompson (1959) we have found no vitamin A aldehyde in *Calanus finmarchicus*. All our evidence indicates that copepods are not a source of preformed vitamin A for euphausiids feeding on them. Earlier results show that the main carotenoid in copepods is astaxanthin possibly with small quantities of β-carotene. In the recent chromatographic analysis of extracts from *C. finmarchicus*, however, using the gradient elution method, we obtained a pigment, or pigments, which exhibited a complex absorption spectrum with three peaks all below 400 mμ. This pigment will be the subject of further study and its possible significance as a provitamin A will be investigated. Previously we demonstrated that the most striking increase in both the absolute amount and the concentration of vitamin A in *Meganyctiphanes norvegica* occurred during the spring period (Fisher *et al.*, 1954) just at the time when, as we have now confirmed, they feed mainly on copepods which contain no obvious provitamin or vitamin A, rather than at other times of year when they are eating the eyes of eucaridan Crustacea which we know to be rich in the vitamin. In laying stress on the absence of compound eyes from the stomachs during the spring feeding period (see Figs. 3 and 4), we realize that our 1958 results did not show this absence. In 1957, however, compound eyes were absent from the stomachs only in the second April and the May hauls, which were taken during the period of 74 days between the first April and the June hauls. In 1958 no hauls were taken over the 78 days that elapsed between the hauls in April and in June. Thus we may well have missed the period corresponding to that in 1957 when no compound eyes were found in the stomachs and the euphausiids were feeding on copepods. Moreover, *Calanus finmarchicus* was not caught in large numbers in the area until 13 May in 1958, which is much later for its appearance than is normal; in 1957, it became abundant between the first and second collecting trips in April.

We have suggested astaxanthin derived from copepods as a possible precursor for vitamin A in the euphausiids (Fisher *et al.*, 1954; Kon, 1954) but this pigment is present not only in copepods but in all crustaceans, especially in their eyes (Fisher *et al.*, 1952). If astaxanthin is indeed the precursor euphausiids obtain from copepods it must be in them in a more available form than in other crustaceans to account for the more rapid increase in the vitamin A reserves of euphausiids when they feed almost exclusively on copepods. In any event, the vitamin A they derive from eating other Eucarida is unlikely to account for all the reserves built up in *Meganyctiphanes norvegica*. Some additional source must exist.

After detritus and Crustacea, dinoflagellates appear to us to be the next most important component of the diet of *M. norvegica*, particularly in the younger adults. In the late summer and early autumn dinoflagellates reach their maximum incidence in the stomachs, which may be packed full of them. The only information we have about provitamins and vitamin A in dinoflagellates is for the marine species *Prorocentrum micans* (Scheer, 1940) and two species of *Peridinium*, the freshwater *P. cinctum* (Strain, Manning & Hardin, 1944) and the marine *P. trochoideum*[1]. There is no information about the vitamin itself in *Prorocentrum micans* or *Peridinium cinctum*; it was absent from *P. trochoideum.* In all three species the carotenoids included both carotenes and xanthophylls. In *Prorocentrum micans* about 10% of the carotenoids were carotenes and the rest presumably xanthophylls. The carotenes were almost entirely β-carotene in *Peridinium cinctum* and *P. trochoideum* in quantities greater than in diatoms. As in *Prorocentrum micans* most of the carotenoids in *Peridinium* were xanthophylls at a concentration higher than in most diatoms (Strain *et al.*, 1944). The three main xanthophylls separated from extracts of *P. cinctum* were peridinin (first detected in *Peridinium* spp. by Kylin (1927)), dinoxanthin and diadinoxanthin, of which the first was the most abundant (Strain *et al.*, 1944). The chemical properties of these xanthophylls are still unknown, but they are unlikely to be provitamins A. β-carotene from dinoflagellates is, however, undoubtedly an important seasonal precursor of the vitamin for *Meganyctiphanes norvegica.*

Although we agree with Einarsson (1945) and Ponomareva (1955) that diatoms do not form an important component of the diet of *M. norvegica*, those diatoms eaten will contribute to the carotenoids taken up by the euphausiid. Vitamin A itself does not occur in those species in which it has been sought, namely *Coscinodiscus concinnis*, *Skeletonema costatum* and *Thalassiosira gravida*[1]. The main carotene was β-carotene which formed up to about 5% of the total carotenoids. It was the principal carotene in several other species studied by Strain *et al.* (1944). These workers also characterized the xanthophylls. The most abundant was fucoxanthin and of the others the most important were diadinoxanthin and diatoxanthin. Goodwin (1952) has suggested that these two pigments may be *cis*-isomers of lutein and zeaxanthin respectively. If so, neither of them is a provitamin A. Zeaxanthin has the same molecular structure as β-carotene with the addition of an hydroxyl group to each of its β-ionone rings and could be an intermediate between β-carotene and astaxanthin, which has the structure of zeaxanthin with a ketone group in each β-ionone ring. It is possible, therefore, that diatoxanthin may be a connecting link between the important plant carotenoid, β-carotene, in the phytoplankton and the equally important animal carotenoid, astaxanthin, in the crustaceans feeding on it.

Filamentous algae were a constant, if only minor, constituent of the food of

---

[1] Fisher, L. R., London Univ. Ph.D. thesis, 1953.

Meganyctiphanes norvegica. Most of those eaten were probably green algae with carotenoids similar to those of land plants, possessing β-carotene as their principal vitamin A precursor.

Young *M. norvegica* have in their food several sources of β-carotene, namely dinoflagellates, vegetable detritus of terrestrial origin, algae and diatoms. It is probable that they convert β-carotene into vitamin A like higher animals. As they grow larger they become increasingly carnivorous, feeding more and more on Crustacea, although still taking dinoflagellates when these are abundant. The Crustacea provide them with preformed vitamin A when eucaridan species are eaten, but in the spring copepods are eaten almost exclusively and their principal carotenoid is astaxanthin. It is possible that this carotenoid may be converted into vitamin A just as it is said to be by poeciliid fish (Grangaud & Massonet, 1955). Evidence of the rapid accumulation of the vitamin at a time when the euphausiids are feeding on copepods supports this hypothesis (Fisher *et al.*, 1954).

The fern sporangia found in small numbers in the stomachs of *M. norvegica*, indicate that there may be an inkling of truth in the long-held belief that the bracken-clad hills bordering the loch nourish the Loch Fyne herring, one of the predators of *M. norvegica*, and impart to them and the kippers made from them their renowned high quality (Kerr, 1928, 1949; McCallien, 1938). Macdonald (1927) also discussed the dependence of the herring, through *M. norvegica*, on the abundance of terrigenous vegetable detritus. Essentially the reasoning is correct but we now know that the food chain is longer and more complex, involving both organic and inorganic nutrients from the land. The phytoplankton flourishes on the former and the zooplankton feeds, in turn, on it as well as on the land-derived vegetable material with its carotenoids, especially β-carotene. Such a diet produces specimens of *M. norvegica* in Loch Fyne larger than any we have seen from other waters, including the North Sea, the north Atlantic and the Mediterranean; these large animals are much richer in vitamin A than those we have analysed from the other localities mentioned (Fisher *et al.*, 1955).

Facilities for collecting the material were very kindly provided by the Director of the Marine Station at Millport and we are very grateful to him and his staff, especially the skippers and crews of M.V.'s 'Calanus' and 'Mizpah'. For much helpful discussion and criticism during the preparation of this paper we are indebted to Drs S. M. Marshall, S. K. Kon, J. Mauchline and A. P. Orr.

SUMMARY

Feeding intensity in *Meganyctiphanes norvegica* in Loch Fyne reached its peak during the spring plankton outburst, falling slightly during the summer, to be renewed in the early autumn and decreasing to a minimum in November.

There was a well-defined period of increased feeding from December to February, followed by another decrease in March.

The main food items in decreasing order of importance were organic debris and inorganic detritus, Crustacea, dinoflagellates, diatoms, algae, fern sporangia and dipteran egg membranes. Organic and inorganic material was taken at all seasons, although possibly in smaller amounts during the spring feeding period, when the euphausiids spend more time actively swimming.

Crustacean material occurred most commonly in the stomachs in the spring, when the absence of compound eyes indicated that it was probably all of copepod origin. These eyes, mostly euphausiid, occurred frequently in the stomachs from June onwards and during the late winter.

Dinoflagellates predominated in later summer when some stomachs were packed with them. Diatoms were most numerous during the spring but were never eaten to the same extent as the dinoflagellates. Fern sporangia and dipteran egg capsules appeared in the stomachs in the spring and summer months. Algae were eaten by a few animals at all seasons.

Size of the animal and feeding intensity showed no apparent relationship. The percentage of stomachs containing organic and inorganic particles increased with the size of the animal. Crustacean remains also occurred more frequently in larger animals, but less commonly in the largest specimens of all. Dinoflagellates were eaten by all sizes, with the highest incidence in those of intermediate size.

No consistent differences were observed between the intensities of feeding by day and at midnight. When feeding was intensive, especially in the spring, the relative uptake of organic and inorganic material was lower than during periods when feeding was on a reduced scale.

When the dinoflagellates were abundant in the plankton they occurred in large numbers at all times, but, when they were scarcer, the numbers of *Ceratium*, *Dinophysis* and *Peridinium* tended to be higher in stomachs by day than by night and those of *Prorocentrum* and *Phalacroma* by night.

Samples of mud taken at a depth of 175 m from the bottom of Loch Fyne contained no measurable vitamin A. The amount of carotenoid pigments and of β-carotene increased with the distance below the surface.

Preformed vitamin A is present in compound eyes and its precursor, β-carotene, in dinoflagellates, diatoms, algae and fern sporangia, all of which are eaten by *Meganyctiphanes norvegica*, and so contribute to its high vitamin A reserves. In the spring, however, when the food consists almost entirely of copepods with astaxanthin as their main, if not only, carotenoid, vitamin A concentrations increase most rapidly. It is possible that astaxanthin is a vitamin A precursor in euphausiids.

REFERENCES

Barnes, H., & Goodley, E. F. W., 1958. A note on rainfall in the west of Scotland. *Glasg. Nat.*, Vol. 18, pp. 45–54.

Bigelow, H. B., 1914. Oceanography and plankton of Massachusetts Bay and adjacent waters, November 1912–May 1913. *Bull. Mus. comp. Zool. Harv.*, Vol. 58, pp. 385–419.

Einarsson, H., 1945. Euphausiacea. I. Northern Atlantic species. *Dana Rep.*, No. 27, 185 pp.

Euler, H. von, Hellström, H. & Klussmann, E., 1934. Über den Carotenoidgehalt einiger Evertebraten. *Hoppe-Seyl. Z.*, Bd. 228, pp. 77–89.

Fisher, L. R. & Kon, S. K., 1959. Vitamin A in the invertebrates. *Biol. Rev.*, Vol. 34, pp. 1–36.

Fisher, L. R., Kon, S. K. & Thompson, S. Y., 1952. Vitamin A and carotenoids in certain invertebrates. I. Marine Crustacea. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 229–58.

—— —— —— 1954. Vitamin A and carotenoids in certain invertebrates. II. Studies of seasonal variations in some marine Crustacea. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 589–612.

—— —— —— 1955. Vitamin A and carotenoids in certain invertebrates. III. Euphausiacea. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 81–100.

Fox, D. L., 1937. Carotenoids and other lipid-soluble pigments in the sea and in deep marine mud. *Proc. nat. Acad. Sci., Wash.*, Vol. 23, pp. 295–301.

—— 1950. Comparative metabolism of organic detritus by inshore animals. *Ecology*, Vol. 31, pp. 100–8.

Fox, D. L. & Anderson, L. J., 1941. Pigments from marine muds. *Proc. nat. Acad. Sci., Wash.*, Vol. 27, pp. 333–7.

Fox, D. L., Isaacs, J. D. & Corcoran, E. F., 1952. Marine leptopel, its recovery, measurement and distribution. *J. mar. Res.*, Vol. 11, pp. 29–46.

Fox, D. L., Updegraff, D. M. & Novelli, D. G., 1944. Carotenoid pigments in the ocean floor. *Arch. Biochem.*, Vol. 5, pp. 1–23.

Goodwin, T. W., 1952. *The Comparative Biochemistry of the Carotenoids.* 356 pp. London: Chapman and Hall.

Grangaud, R. & Massonet, R., 1955. Recherches sur la biogénèse de la vitamine A des poissons. I. L'astaxanthine provitamine A pour *Gambusia holbrooki* Grd. *Arch. Sci. physiol.*, T. 9, pp. 245–56.

Hickling, C. F., 1923–5. Notes on euphausiids. *J. mar. biol. Ass. U.K.*, Vol. 13, pp. 735–45.

Holt, E. W. L. & Tattersall, W. M., 1905. Schizopodous Crustacea from the north-east Atlantic slope. *Rep. Sea Inl. Fish. Ire., Sci. Invest.*, Year 1902–3, App. 4, pp. 99–152.

Kerr, J. G., 1928. *General Handbook of the British Association*, pp. 347–57.

—— 1949. The Scottish Marine Biological Association. *Notes roy. Soc. Lond.*, Vol. 7, pp. 81–96.

Kon, S. K., 1954. Vitamine A des invertébrés marins et métabolisme des caroténoïdes du plancton. *Bull. Soc. Chim. biol., Paris*, T. 36, pp. 209–25.

Kylin, H., 1927. Über die karotinoiden Farbstoffe der Algen. *Hoppe-Seyl. Z.*, Bd. 166, pp. 39–77.

Lederer, E., 1938. Recherches sur les caroténoïdes des invertébrés. *Bull. Soc. Chim. biol., Paris*, T. 20, pp. 567–610.

Macdonald, R., 1927. Food and habits of *Meganyctiphanes norvegica*. *J. mar. biol. Ass. U.K.*, Vol. 14, pp. 753–84.

MAUCHLINE, J., 1959. The biology of a euphausiid crustacean, *Meganyctiphanes norvegica* (M. Sars). *Proc. roy. Soc. Edinb.* (in the Press).

MCCALLIEN, W. J., 1938. *Geology of Glasgow and District.* 190 pp. London and Glasgow: Blackie.

MORTIMER, C. H., 1942. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.*, Vol. 30, pp. 147–201.

PAULSEN, O., 1909. Plankton investigations in the waters round Iceland and in the north Atlantic in 1904. *Medd. Komm. Havundersøg., Kbh.*, Ser. Plankton, Bd. 1, No. 8, 57 pp.

PLACK, P. A., KON, S. K. & THOMPSON, S. Y., 1959. Vitamin A_1 aldehyde in the eggs of the herring (*Clupea harengus* L.) and other marine teleosts. *Biochem. J.*, Vol. 71, pp. 467–76.

PLACK, P. A., THOMPSON, S. Y. & KON, S. K., 1958. Vitamin A_1 aldehyde in fish eggs. *Biochem. J.*, Vol. 68, p. 2*P*.

PONOMAREVA, L. A., 1955. The feeding and distribution of euphausiids of the Sea of Japan. *Zool. Zh.*, Vol. 34, pp. 85–97. (In Russian.)

POULSEN, E. M., 1926. Om den store Lyskrebs (*Meganyctiphanes norvegica*) Betydning som Fiskeføde i Skagerak. *Dansk. Fisk-Tid.*, Bd. 24, pp. 286–9.

RUUD, J. T., 1926. Zooplankton of the coast of Möre, 1926. *Rapp. Cons. Explor. Mer*, Vol. 41, pp. 120–4.

SCHEER, B. T., 1940. Some features of the metabolism of the carotenoid pigments in the California sea-mussel (*Mytilus californianus*). *J. biol. Chem.*, Vol. 136, pp. 275–99.

STRAIN, H. H., MANNING, W. M. & HARDIN, G., 1944. Xanthophylls and carotenoids of diatoms, brown algae, dinoflagellates and sea anemomes. *Biol. Bull., Woods Hole*, Vol. 86, pp. 169–91.

STUART, T. A., 1945. Chironomid larvae of the Millport shore pools. *Trans. roy. Soc. Edinb.*, Vol. 60, pp. 475–502.

J. mar. biol. Ass. U.K. (1959) 38, 313–318
Printed in Great Britain

A LINEAR PATTERN ON THE SEA FLOOR AND ITS INTERPRETATION

By A. H. Stride

National Institute of Oceanography, Wormley, Surrey

(Plate I and Text-fig. 1)

The redistribution of sediments by tidal streams has been demonstrated in a number of ways in the seas around Britain. For example, the numerous banks in the North Sea are elongated parallel to the streams, while the depth and position of certain channels are subject to such important changes in position that re-surveys have to be made each year or so (Robinson, 1956). In the same region the streams, reaching about a knot or more at the surface, have wrought a large area of sand floor into ridges normal to their path. Off the Dutch coast the grade of the sand (Jarke, 1956) decreases in their inferred direction of advance (Stride & Cartwright, 1958). Similar relief is found even at 90 fathoms near the edge of the continental shelf, at the western approaches to the English Channel (Cartwright & Stride, 1958). Pratje (1950) has shown that the occurrence and grade of loose sediment on the floor of the Channel as a whole is directly related to the velocity of the streams overhead.

Fresh evidence can now be given of the action of tidal streams on some flat floors of the English Channel and North Sea.

SURVEY

During April 1958 a survey of the floor (Fig. 1) was made by R.R.S. 'Discovery II' in the large open bay between Start and Dodman Points, near Plymouth, using echo-ranging equipment such as described by Chesterman, Clynick & Stride (1958). The present equipment had the following characteristics: frequency 37 kc/s; pulse length 1 ms; beam shape 1·8° (horizontal), 11° (vertical), with four side lobes in addition; tilt angle 5–7° below horizontal. A sample of the records obtained is shown in Pl. I, with the range abeam of 732 m (800 yards) as the horizontal scale.

During September 1958 five cores and photographs of the floor were taken by R.V. 'Sarsia' at positions shown near P in Fig. 1.

THE LINEAR PATTERN

The pattern has been recognized in a curved belt about 7 miles wide, roughly parallel to the Cornish coast from between Rame Head and Eddystone to Dodman Point. It does not extend as far east as the line run north-east from

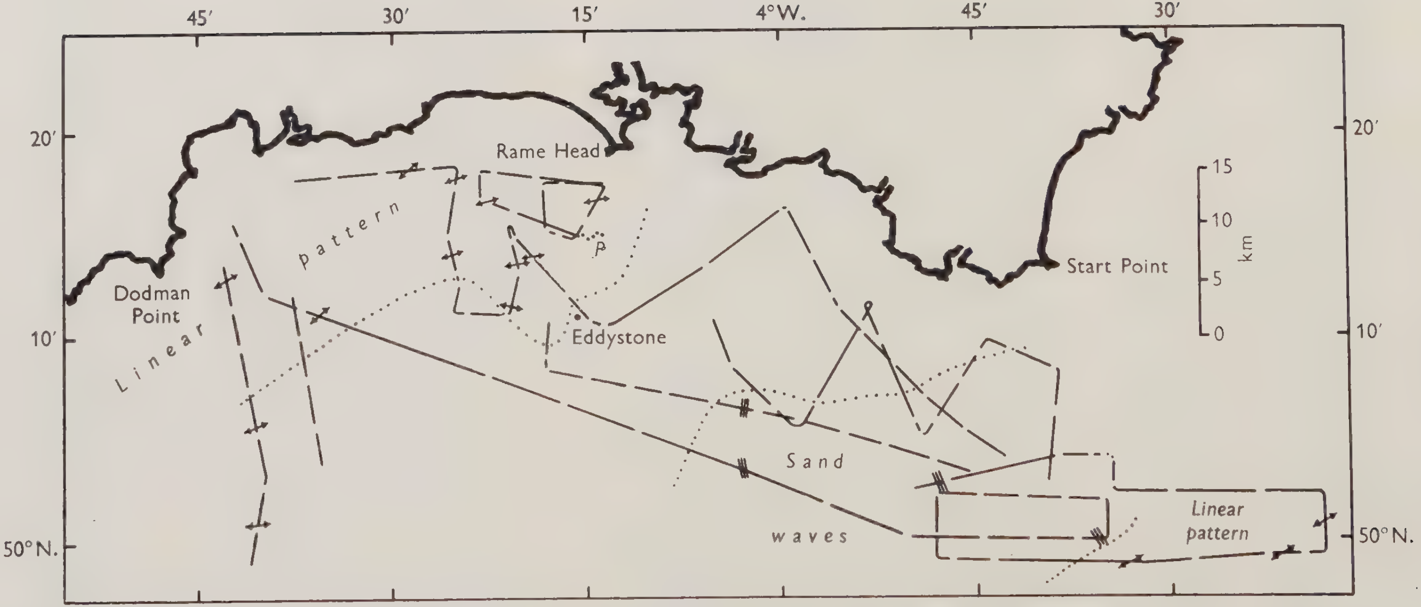

45′
30′
15′
4°W.
45′
30′
20′
10′
50°N.
Rame Head
Start Point
Dodman Point
Eddystone
Linear pattern
Sand waves
15
10
5
0
km
P

Fig. 1. Courses on which the acoustic survey was made are shown by thick lines. The patches were elongated parallel to the arrows and the crests of sand waves lay parallel to the groups of 3 lines.

Eddystone. The southern margin is gradational; the south-western limit has not yet been found. The floor is virtually devoid of relief except for the small regional depth gradient.

The acoustic pattern consists of well defined, almost parallel patches of contrasting reverberation level, such as extend across Pl. I. The low reverberation patches certainly range up to 1·8 km in length and between 50 and 200 m wide and about the same distance apart. In the middle of the region the patches are wider. Some patchiness is found farther south from here and may be present south of Start Point.

Off the East Anglian coast the patches are locally well developed, certainly reaching 1·4 km in length, yet only 90 m in width. The boundaries are generally sharp, the ends are tapered.

In each place the patches are elongated parallel to the path of the strongest tidal streams. For the Plymouth region the observed orientation of the patches is shown by the arrows in Fig. 1. The patches south of Start Point are orientated normal to the crests of sand waves occurring nearby, themselves an indicator of the direction of the streams (Chesterman *et al.*, 1958). The sand waves are up to 3 m high and mostly between 100 and 250 m apart. They are smallest and least well developed at the margins of the patch.

TABLE 1. LITHOLOGY OF CORES TAKEN NEAR EDDYSTONE

Core no.	Layer thickness (cm)	Lithology	Cumulative weight percentage of coarse fractions		
			> 12 mm	> 6 mm	> 2 mm
1	16	Fine sand with shell and rock fragments. Trace of coal	3	10	25
	10	Coarse reddish shell 'gravel' with about 30 % sand. Boundary gradational	—	—	—
2	1	Fine sand on surface	0	0	5
	13	Fine sand with shell and rock fragments. Trace of coal	—	—	—
	10	Coarse 'gravel' of shell and rock debris	—	—	—
3	13	Fine sand with shell fragments. Trace of coal	0	2	10
	4	Coarse 'gravel' of shell and rock debris with about 20 % sand	—	—	—
4	3	Fine sand with shell and rock debris	0	4	16
	12	Same as above with trace of mud	—	—	—
	8	Coarse gravel of shell and rock debris up to 2 cm diameter, with about 20 % sand	—	—	—
5	2	Fine sand	0	0	10
	16	Sand at top grading down into gravel	—	—	—
	4	Fine gravel of shell and rock debris	—	—	—

COMPOSITION OF THE FLOOR

Between Eddystone and Plymouth grab sampling has revealed that the floor may be made of almost pure sand or sand with an appreciable fraction of coarser material, greater than 2 mm in diameter (Holme, 1953).

Five cores were taken by gravity corer in this patchy floor at the positions shown in Fig. 1 and are described in Table 1 (uppermost bed first in each case).

The cores reached from 17 to 26 cm below the surface. The base of core 1 was red in colour, rather suggesting that bed rock was nearly reached, although Holme (1953) found more than 70 cm of superficial sediments nearby.

A coarse gravel, consisting mainly of fragments of large shells with subordinate sand and rock debris, was found at the base of cores 1–4, while in core 5 there was a well-graded fine gravel.

Above the basal bed there was up to 16 cm of sand carrying as much as 25% of particles between 2 and 14 mm in diameter. In cores 2 and 5 these sediments were capped by a thin layer of sand. The abundance of the coarse fractions in the surface layers is shown in Table 1, although the figures are misleading as the particles are largely platey shell fragments.

Three samples, taken by Van Veen grab, from similar patch floor near the East Anglian coast consisted of the following components:

Sample	Sand (%)	Gravel and stones (%)
1	10	90
2	60	40
3	70	30

The first sample showed that the floor was locally made of gravel and stones carrying polyzoa and hydroids. These were absent from the second sample so that the coarse fraction was probably covered by sand. In the third case the floor proved to be made largely of sand.

DISCUSSION

The acoustically patchy floor must be an expression of differences in composition since at both localities the floor was flat. The patches of low reverbation level should correspond to the patches of sand while the high reverberation level should be caused by the presence of coarser sediments. This correlation can be made with confidence for the East Anglian example because of the considerable difference in the grade of the sediments (Chesterman *et al.*, 1958). There is good reason to believe that in the surface sediments of core 1, of the Plymouth region, there is sufficient coarse, platey material to make the sediment readily distinguishable acoustically from the surface sands of cores 2 and 5. The uppermost material of the remaining cores is probably representative of the high reverberation patches also. The larger area of acoustically patchy floor, which extends to the west of the region sampled, must be due to similar types of lithological contrast.

The acoustic pattern is interpreted as representing patches of sand lying on either pebbles or sand with up to 25% shell fragments between 2 and 12 mm wide. The patches of sand are longer than broad. The ratio is largest off the east coast where the streams reach more than 2 knots.

In both localities the streams are virtually linear, flowing to-and-fro parallel to the length of the patches. There is a difference of more than 0·1 knot between the ebb and flood streams so that the sand is probably being driven into the two localities.

The growth of a similar but smaller pattern has been seen during sand storms in a desert by Bagnold (1954, p. 176) and in a flume (Casey, 1935) under an unidirectional stream. In a desert the parallel sand strips average ½ km in length (replacing each other *en échelon*), are 1–3 m wide, 1–2 cm thick and are separated by 40–60 m of flat, pebble-covered ground. They appear to be initiated when a strong sand-laden wind blows over a uniformly rough surface (of pebbles, say) due to lateral instability of the flow, and grow so long as the drag over the sand exceeds that over the pebbles.

The author wishes to thank M. J. Tucker and A. R. Stubbs for designing their fish-detection equipment so as to make it suitable for surveying the sea floor. To these and to other colleagues the author is grateful for the help they gave in taking the records and for valuable discussion. Professor W. F. Whittard kindly made it possible to take the five cores and occupy the camera station during his cruise on R.V. 'Sarsia'.

SUMMARY

An acoustic survey has been made of two regions where there is marked geographical variation in the nature of the surface sediments. It is shown that the patches are elongated parallel to the prevailing path of the tidal streams and a mechanism of origin is suggested.

REFERENCES

Bagnold, R. A., 1954. *The Physics of blown Sand and Desert Dunes.* London: Methuen.

Cartwright, D. & Stride, A. H., 1958. Large sand waves near the edge of the continental shelf. *Nature, Lond.*, Vol. 181, p. 41.

Casey, H. J., 1935. Über Geschiebebewegung. Dissertation der Technischen Hochschule, Berlin.

Chesterman, W. D., Clynick, P. R., & Stride, A. H., 1958. An acoustic aid to sea bed survey. *Acustica*, Vol. 8, pp. 285–290.

Holme, N. A., 1953. The biomass of the bottom fauna in the English Channel off Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 32. pp. 1–49.

Jarke, J., 1956. Der Boden der südlichen Nordsee. *Dtsch. hydrogr. Z.*, Bd. 9, pp. 1–9.

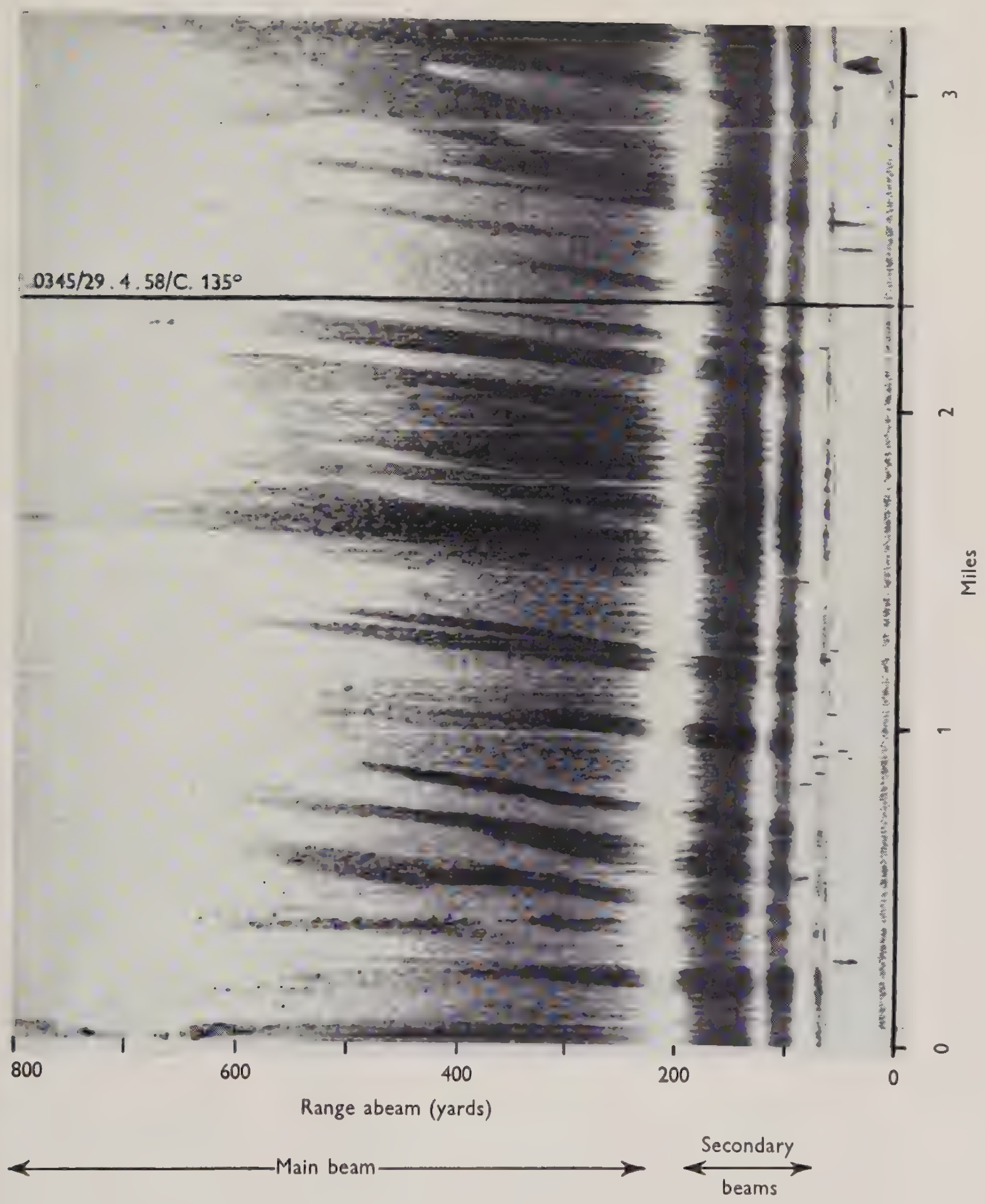
Pratje, O., 1950. Die Bodenbedeckung des Englischen Kanals und die maximalen Gezeitenstromgeschwindigkeiten. *Dtsch. hydrogr. Z.*, Bd. 3, pp. 201–205.

Robinson, A. H. W., 1956. The submarine morphology of certain port approach channel systems. *J. Inst. Navig.*, Vol. 9, pp. 20–46.

Stride, A. H. & Cartwright, D., 1958. Sand transport at southern end of North Sea. *Dock Harb. Author.*, Vol. 38, pp. 323–324.

EXPLANATION OF PLATE I

Plate I. An acoustic map of patches on the floor near Eddystone. Range abeam of ship of 800 yards is exaggerated × 7 distance scale. The light toned patches extending diagonally across the record are associated with patches of sand.


0345/29.4.58/C. 135°
3
2
Miles
1
0
800
600
400
200
0
Range abeam (yards)
Main beam
Secondary
beams

J. mar. biol. Ass. U.K. (1959) 38, 319–333
Printed in Great Britain

ELECTRON MICROSCOPICAL OBSERVATIONS ON A VERY SMALL FLAGELLATE: THE PROBLEM OF *CHROMULINA PUSILLA* BUTCHER

By Irene Manton

Botany Department, Leeds University

(Plates I–X)

CONTENTS

Introduction 319
Material and methods 320
External morphology 321
Internal structure 323
  The flagellum 323
  The nucleus 324
  The plastid 325
  The mitochondrion 325
  Other cytoplasmic components 326
Division stages 327
Discussion 329
Summary 332
References 332

INTRODUCTION

The marine plankton flagellate hitherto known as *Chromulina pusilla* Butcher is one of the smallest of algal cells, being of bacterial dimensions (1–1·5μ, Butcher, 1952). For this reason it had seemed of interest to investigate its structure with the electron microscope as part of a general programme on ciliated cells in plants which has been in progress for some years. It soon became apparent, however, that there were more points of importance than had been expected at the outset. In particular the anatomical findings showed that the formal taxonomic description based on the light microscope was not only incomplete (an unavoidable consequence of the very small size), but that, as a further consequence of these physical limitations, it contained some rather fundamental misconceptions regarding the taxonomic position of the organism. By the kindness of correspondents and collaborators it was fortunately possible at once to follow up the anatomical evidence biochemically, and the combined data make the conclusion unavoidable that this organism is not a member of the Chrysophyceae (to which group the genus *Chromulina* properly belongs) but that its affinities lie in or near the Chlorophyceae. This naturally raises some difficult nomenclatural questions which cannot all be immediately resolved. The anatomical facts for this particular species are not, however, in themselves dependent on nomenclature, and since this organism is not merely an electron microscopical curiosity but is also exceedingly abundant[1] in coastal waters near the British Isles, it is perhaps of some value to students of plankton that they should be made available.

I am indebted to my former technical assistant Mr B. Clarke for carrying out all the work involving shadow-cast whole mounts for external morphology, including the taking of the first four micrographs assembled on Pl. I, and also for photographic help with all the remaining Plates. As donors of material I have to thank Prof. E. W. Knight-Jones in the first instance and Dr M. Parke more recently. I am greatly indebted to the owners of the various microscopes listed below, more particularly to Dr K. R. Porter of the Rockefeller Institute whose help on this, as on many other occasions, it is a pleasure to acknowledge. Finally thanks are due to Dr G. Y. Kennedy of Sheffield for providing the chemical data quoted on p. 331 and to those members of the staff of the Marine Biological Association's Laboratory at Plymouth involved in making the arrangements and supplying him with the large quantity of material needed.

MATERIAL AND METHODS

We were first introduced to this organism in November 1951 by Dr (now Professor) E. W. Knight-Jones, who had recently isolated it into uni-algal (though not bacteria-free) culture from the Conway estuary (North Wales). We made some preliminary observations on external morphology, the only aspect of structure for which adequate electron microscopical techniques existed at that date, using shadowcast whole mounts of cells killed with osmic vapour directly on to formvar-coated carriers. This isolate had supplied the type material described taxonomically by Butcher (1952) and it was also sent to us again in 1953 from Plymouth as 'flagellate 90'. At the request of Dr M. Parke we made some comparative observations on 'flagellate 90' and a new isolation known as 'flagellate 27' obtained by her from the English Channel which proved to be indistinguishable. Finally a third isolation, made by Dr R. W. Butcher 'in June 1954 from the Thames estuary off Sheerness, Isle of Sheppey, salinity 32‰ at the surface' (Butcher personal communication), was received from Plymouth in 1956 and subsequently, as 'flagellate 90*a*'. Our information on external morphology thus relates to material of unimpeachable authenticity from three different parts of the British coast, namely, Conway estuary (type culture for *Chromulina pusilla*, Plymouth no. 90), Thames estuary (Plymouth no. 90*a*) and English Channel (Plymouth no. 27).

---

[1] Preliminary information on the relative abundance of this organism in estuarine and coastal waters on various parts of the British coasts is given by Knight-Jones (1951) and Knight-Jones & Walne (1951), but it may be of interest to quote a personal statement made by letter to the author by Professor Knight-Jones in September 1958: 'I can sum up by saying that *Chromulina pusilla* was the most abundant organism which I observed and it occurred in the majority of the samples of full salinity sea water, both from enclosed waters and the open sea.'

For the anatomical investigation only flagellates 27 and 90*a* in the Plymouth collection have been used, both supplied to us as required, by Dr M. Parke. Both cultures have been embedded and sectioned several times during 1957 and 1958 but they are so similar that it has not been felt necessary to distinguish them individually in recording the results. Pls. II, III, and V–VIII are of flagellate 27 and Pl. IV, IX and X are of flagellate 90*a*.

The technical processes involved in embedding and also for whole mounts are now standard and need not be quoted in detail. The fixative was 1% osmium tetroxide buffered with acetate veronal to pH 7. All sections were cut in Leeds on a Porter Blum Sorval microtome using a glass knife and they were mounted on carbon films.

Several different microscopes have been used. For external morphology of shadow-cast material, the old Philips microscope in the Leeds Botany Department has been used and is adequate. All sections, however, have been examined on a Siemens microscope. The work was begun on the instrument in the Aeon Laboratories, near Egham, Surrey, during occasional visits in the summer of 1957. It was continued on the Siemens microscope in the Rockefeller Institute, New York, which was made available to me for two weeks at the end of December 1957. Finally it was completed on a similar instrument recently installed in the Leeds Botany Department by means of a grant from the Rockefeller Foundation.

With regard to the chemical data communicated by Dr G. Y. Kennedy of Sheffield, the methods used for the study of the pigments were a modification of those of Willstädter and Stoll 1913, the details of which are being published by Kennedy & Nicol (in the press).

EXTERNAL MORPHOLOGY

The first three figures on Pl. I show all that can easily be seen about the external morphology of the swimming cells by a study of whole mounts. Figs. 1 and 2 represent two of the strains used, at different magnifications. Both cultures are a mixture of the organism and numerous bacteria which occur with it in any field of view. In comparison with the bacteria present, the cells of the flagellate are larger (though not greatly so) and more asymmetrical. When undistorted they are more or less pear-shaped, with a laterally attached flagellum composed of two parts, namely a proximal wider portion of rather less than 1 μ in length and a much more slender distal portion of 2–3 μ long; the lengths of both parts are nevertheless somewhat variable, a matter no doubt associated with changes consequent on the growth cycle (see p. 328).

Apart from the general shape the only other features which can be detected on whole mounts are the striations which run longitudinally down the wider portion of the flagellum and which we now know to be caused by the longitudinally running fibres inside. These striations are just perceptible in the original photograph of the left hand cell in Fig. 1 and again in the more highly magnified flagellum of Fig. 3. Outside the striated portion in Fig. 3 there are faint signs of a covering membrane and the distal tip of the slender extremity ends bluntly.

Explanation of Plates I–IV

Chromulina pusilla Butcher

I

Fig. 1. Shadow-cast whole mount of flagellate 90 in the Plymouth collection, prepared in 1953 and re-examined in 1957 with the Philips microscope, showing 2 cells of the organism together with bacteria; this material was a direct descendant from the type culture used by Butcher 1952 and originally isolated from the Conway estuary by Knight-Jones. Micrograph M 575·4, magnification × 10,000.

Fig. 2. A cell and bacteria from a culture of flagellate 27 in the Plymouth collection, isolated by Dr M. Parke from the English Channel. Micrograph M 514·4, magnification × *c.* 20,000.

Fig. 3. The flagellum of a cell of flagellate 90 more highly magnified. Micrograph M 575·19 × *c.* 30,000.

Fig. 4. A section through a cell of flagellate 27 showing the two parts of the flagellum and internal cell contents which include the plastid and pyrenoid with a part of the nucleus. Micrograph MS 56 × 35,000.

II

Fig. 5. A section showing the plastid with covering membrane, lamellae and pyrenoid (*P*); part of the nucleus (*N*) and cytoplasmic vesicles. Micrograph MS 17, × *c.* 50,000.

Fig. 6. A longitudinal section to show the relative positions of the nucleus (*N*), plastid (*P*), fat body and flagellum. Micrograph MS 61, × *c.* 30,000.

Fig. 7. Longitudinal section (lower cell) to show the positions of the mitochondrion (*m*), plastid (*c*) and golgi vesicles (*xx*), relative to the flagellum. Micrograph MS 30, *c.* 55,000.

III

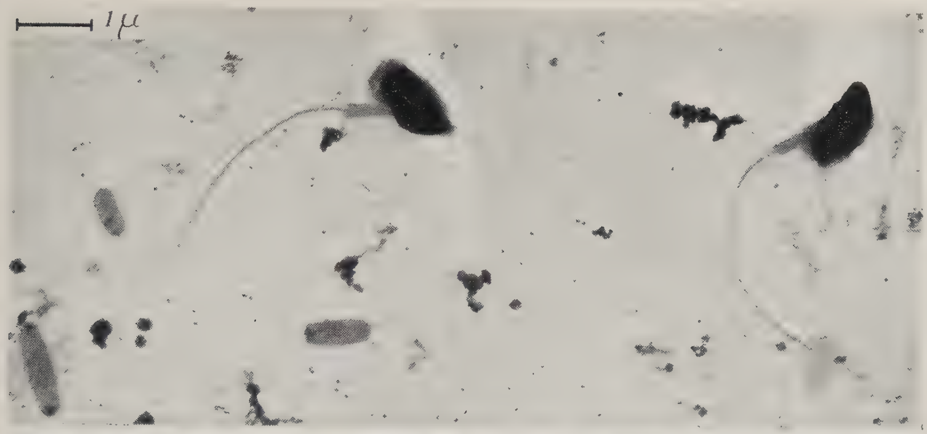
Fig. 8. Longitudinal section showing the flagellar base (*fb*) and arrangement of fibres in the flagellar axis, including the hair-point. Micrograph MS 220 × *c.* 40,000.

Fig. 9. General view of the cell shown at a higher magnification in Fig. 10. Micrograph MS 288 × 18,000.

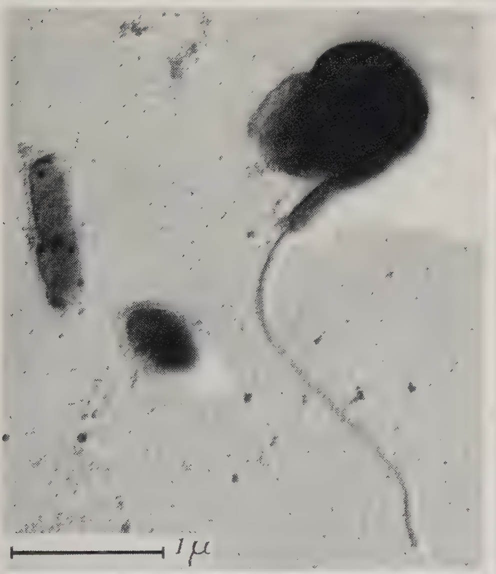
Fig. 10. Part of the cell of Fig. 9 more highly magnified to show the three-layered structure of the membrane covering the hair-point and other details of the arrangement of fibres within the flagellum; the nucleus (*N*), perinuclear space (*ps*), a bacterium in section (*B*). Micrograph MS 291, × 100,000.

IV

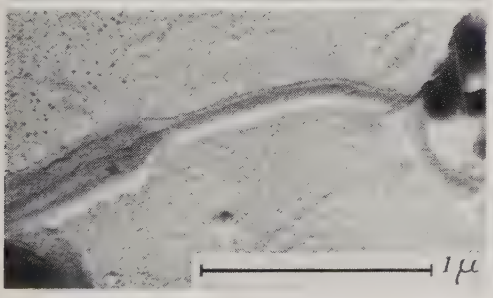
Fig. 11. A cell showing among other features the flagellum, the nucleus (*N*), the mitochondrion in TS (*m*), vesicles (*v*) not part of the peri-nuclear space (*ps*), the plastid with central pyrenoid (*P*), two peripheral small spherical bodies within the plastid and the array of parallel curved membranes near to the plastid surface and joined in pairs at intervals. Micrograph H 1237 × 90,000.



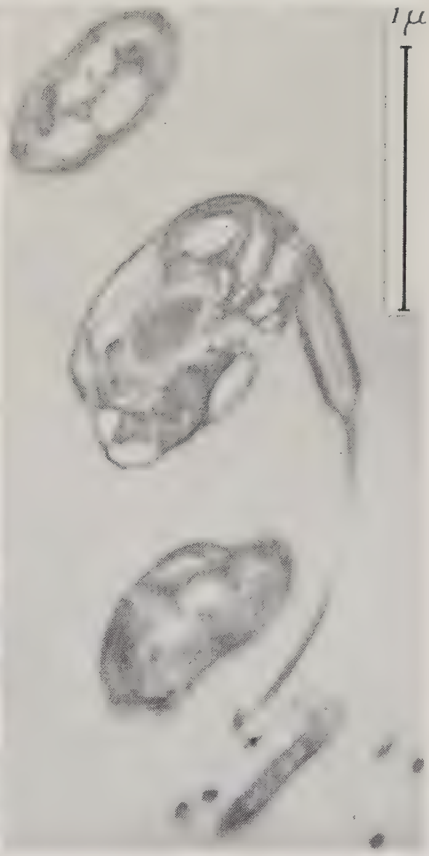
1



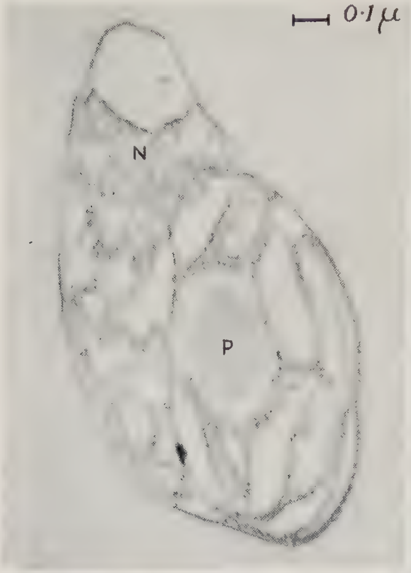
2



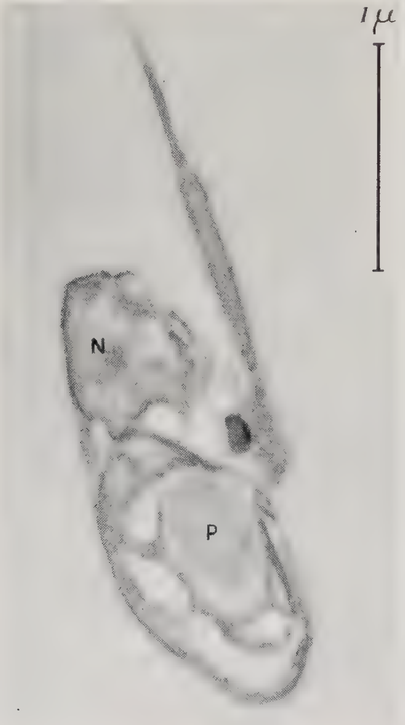
3



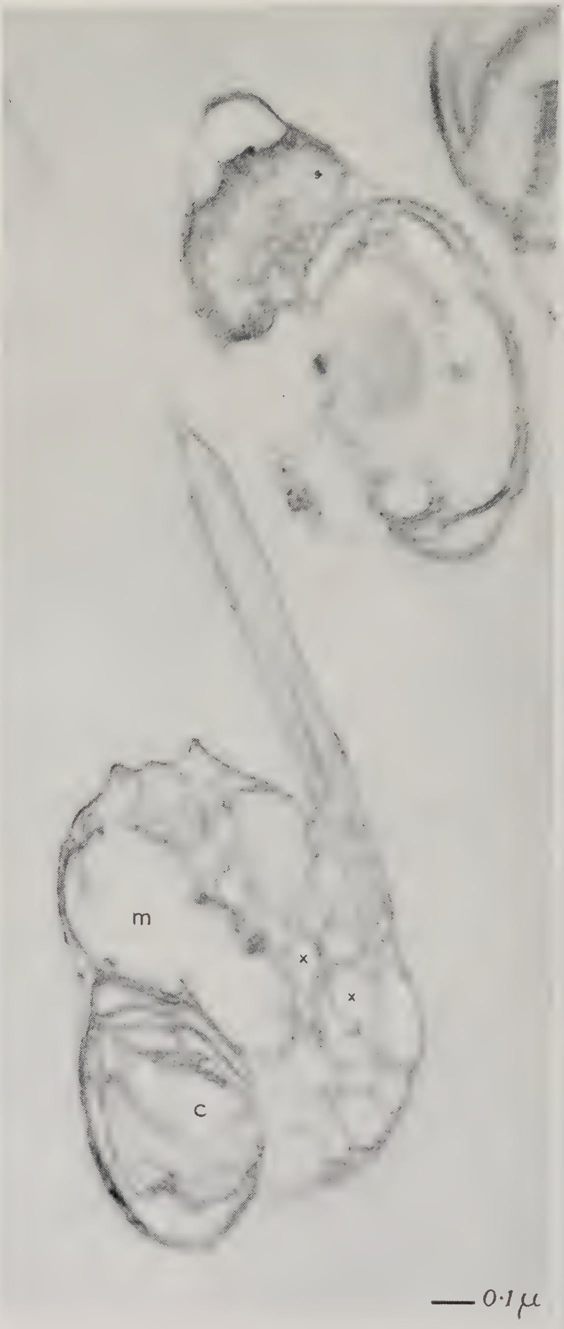
4



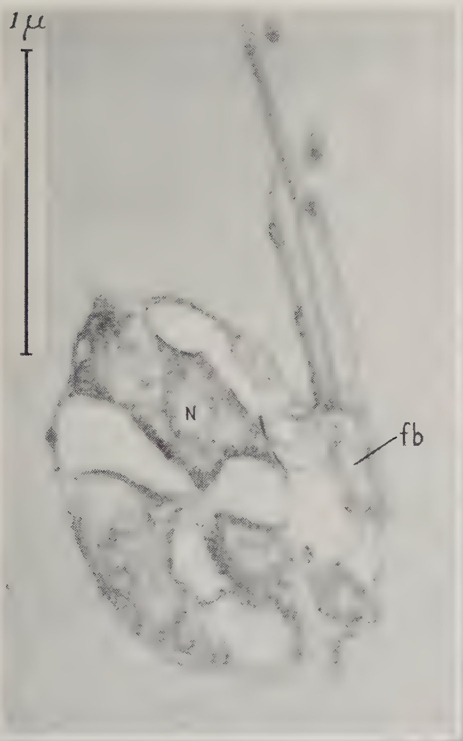
5



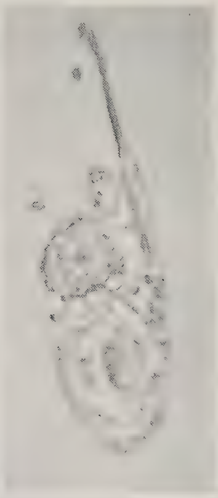
6



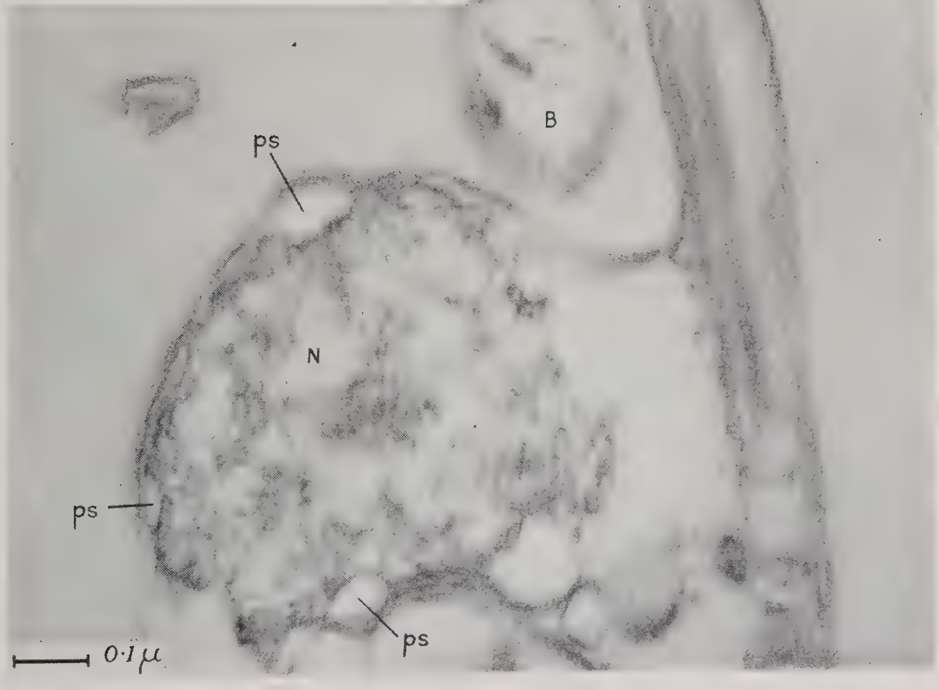
7



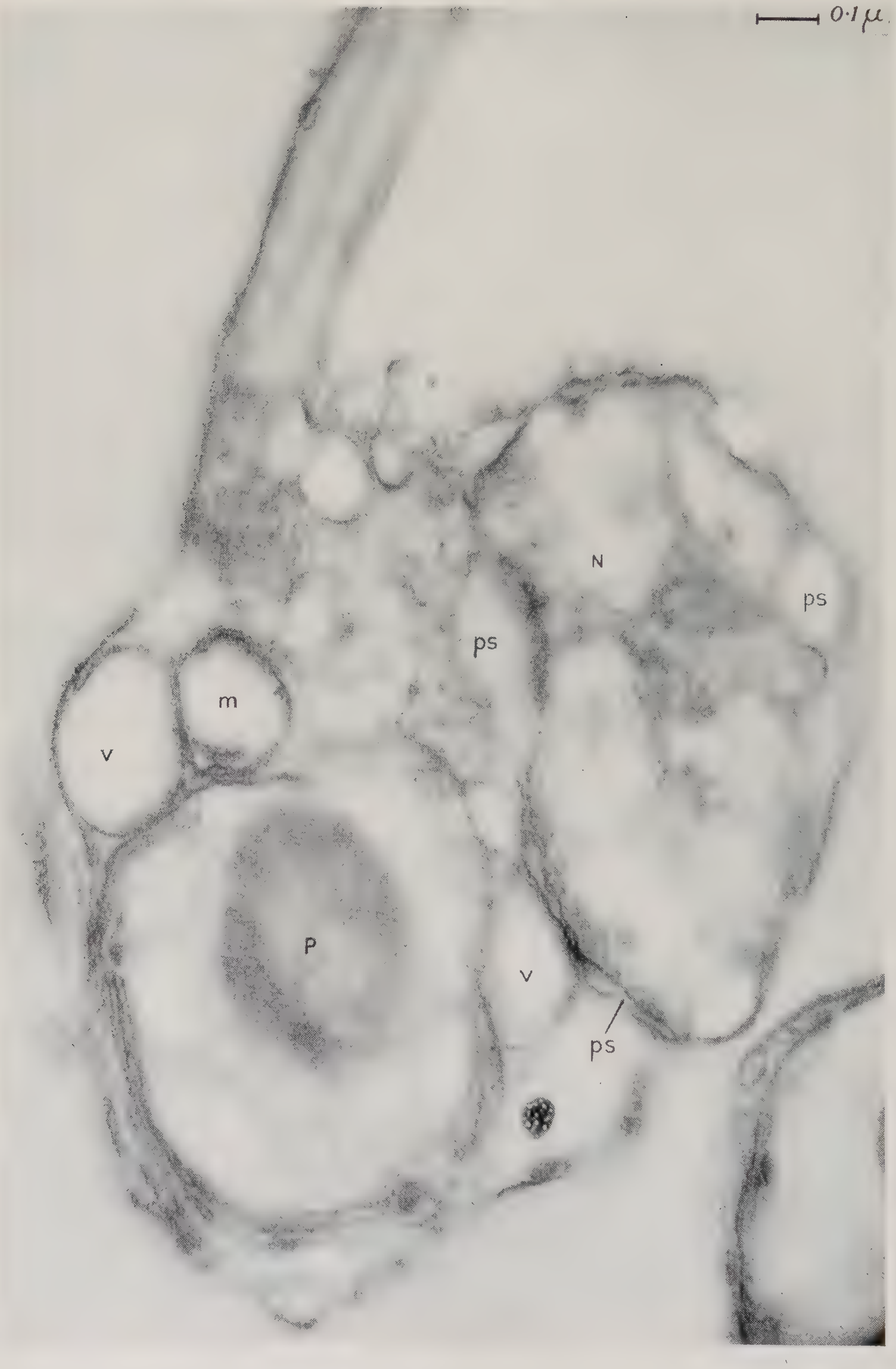
8



9


B
ps
N
ps
ps
0·1μ

10


0·1 μ
N
ps
ps
m
v
P
v
ps

11

INTERNAL STRUCTURE

The flagellum

The first object of enquiry was the internal structure of the two parts of the flagellum since the distal portion is so slender that it seemed unlikely that it could contain the normal 9+2 internal fibres so characteristic of cilia and flagella in plants and animals generally, while the fatter basal part, when first seen, could have been interpreted as a sheath, and this, had it been confirmed, would have been a character of major classificatory importance.

A glance at any of the longitudinal sections included in Pls. I–III is sufficient to dispel any suggestion that the thickening of the base of the flagellum is due to a sheath. The entire surface of both parts is smoothly covered by a membrane which is continuous with the body membrane and, like it, is three-layered, being composed of two electron dense surface layers separated by a translucent central layer. This structure is most clearly demonstrated in the distal extremity of the flagellum shown at a magnification of × 100,000 in Fig. 10, Pl. III, which also shows the smooth tapering which is the only external mark of the transition region between the two parts of the flagellum.

Transverse sections of the basal part of the flagellum appear incidentally in many of the illustrations but most clearly in Plate VI. These show unmistakably that in this region the surface membrane closely covers the normal array of 9+2 strands, among which the outer 9 are double with the plane of separation of half-strands arranged radially. There is therefore nothing peculiar about this part of the flagellum except its extreme shortness.

The structure of the distal region is more difficult to investigate directly for obvious reasons. Transverse sections of it are so small that it is only by accident that one suitably orientated and of sufficient thinness to show internal structure is recorded at a magnification high enough to be of use. One such is however included in Fig. 15, Pl. VI, and if the evidence from this is amplified by comparison with longitudinal views, more especially those of Fig. 8, Pl. III, and Fig. 17, Pl. VII, the general features can be ascertained with reasonable certainty. Within the bounding triple-membrane there seems to be a core composed mainly of prolongations from the two central strands of the basal part of the flagellum, but with little or no contribution from the 9 peripheral strands. The structure is thus not that of a complete flagellum but seems to correspond more to a specialized and exceptionally elongated distal hair-point. Whether or not this hair-point is self-motile or passively dependent on motion transferred from the flagellum proper cannot be directly ascertained, but the apparent simplicity of its structure suggests the latter.

At the base of the flagellum, and separated from it by a transverse diaphragm, there is a normal type of basal body within the cell. The diaphragm is sometimes exactly level with the cell surface, e.g. Fig. 8, Pl. III, but is sometimes much more deeply seated (Fig. 9, Pl. III), a difference of position which must also be connected with the growth cycle and which will be further discussed below (p. 329). When seen in transverse section (Fig. 14, Pl. VI, *fb*, right hand cell) the basal body appears hollow, with a wall composed of 9 peripheral fibres but with no central ones and no separate covering membrane. These are all normal features of flagellar basal bodies.

The nucleus

This is not the most conspicuous organ of the cell but is conveniently dealt with next. It can be seen at a high magnification in Figs. 10 and 11 (Pl. III and IV), after which it will be readily recognized in other parts of many of the remaining plates. The nucleus is somewhat laterally situated within the cell; there is little ascertainable internal structure except a somewhat diffuse nucleolus. (See especially Fig. 12*c* and *d*, Pl. V.)

The nuclear membrane is almost certainly of the usual type though these small cells are ill adapted for clear demonstration of the finer details owing to the strong curvature of the nuclear surface and the difficulty of detecting suitably cut cells. Signs of the perinuclear space bridged at intervals are nevertheless detectable in sections such as those of Figs. 10 and 11, though the extremely large size of certain spaces abutting on the nucleus in other sections (e.g. at one end of the nucleus in Fig. 12, Pl. V, and at both ends in Fig. 13) may be artificial distortions due to stretching of the space and local collapse of the nucleus during the processes involved in embedding.

We have been unable to trace any fibrous or other connexions between the nucleus and the flagellum.

Explanation of Plates V and VI

Chromulina pusilla Butcher

V

Fig. 12*a–e*. Series of consecutive sections through a cell passing through the nucleus (*N*), mitochondrion (*m*), plastid with central pyrenoid (*P*) and peripheral plastid lamellae somewhat distorted by the fixative, a fat body and part of the perinuclear space (*ps*) probably artificially stretched as a fixation artefact. Micrographs RS 162, 161, 160, 158, 157, × *c.* 43,000.

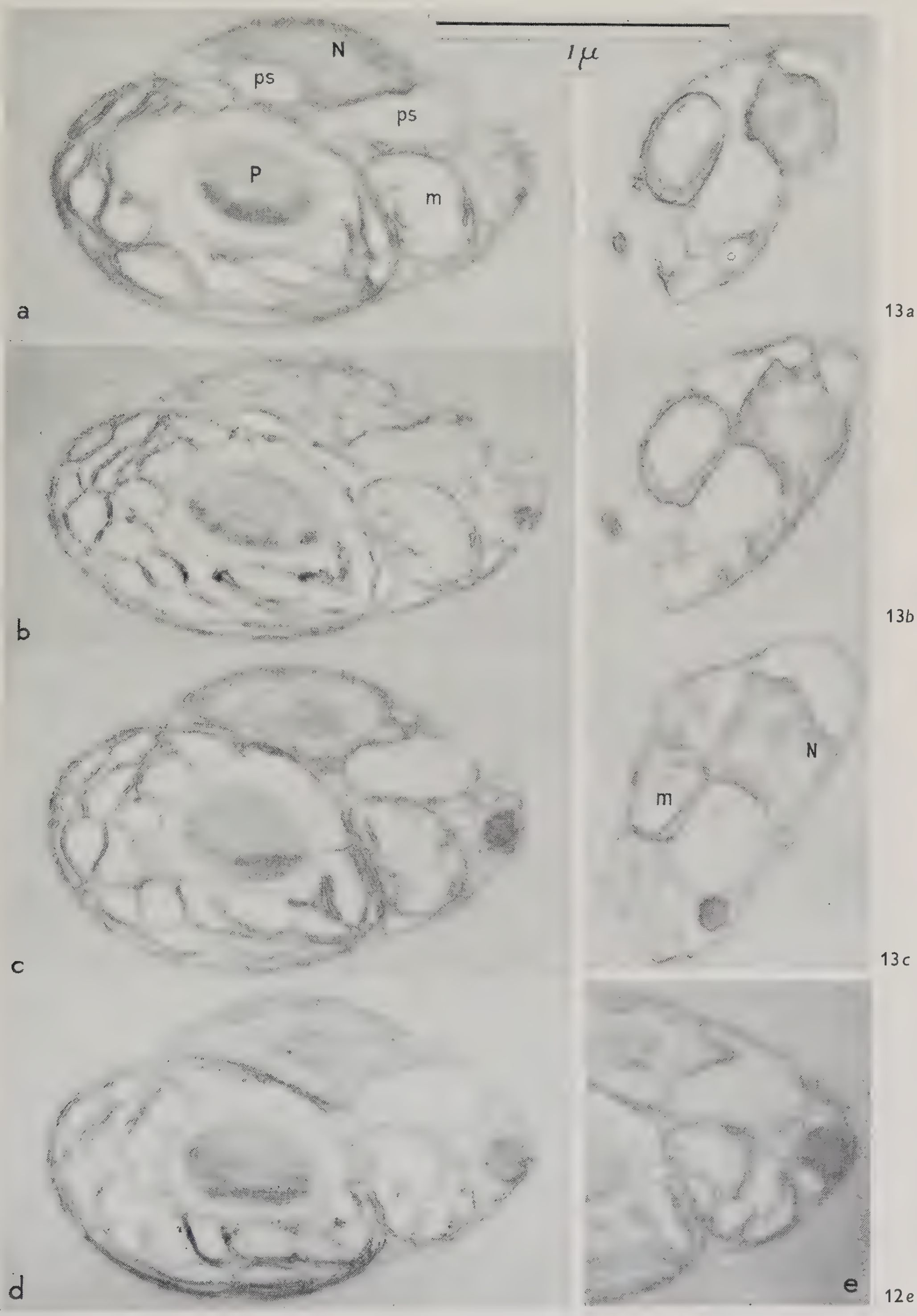
Fig. 13*a–c*. Three consecutive sections through part of another cell to show curvature of mitochondrial cristae; nucleus (*N*), mitochondrion (*m*). Micrographs RS 158, 160, 162, × *c.* 43,000.

VI

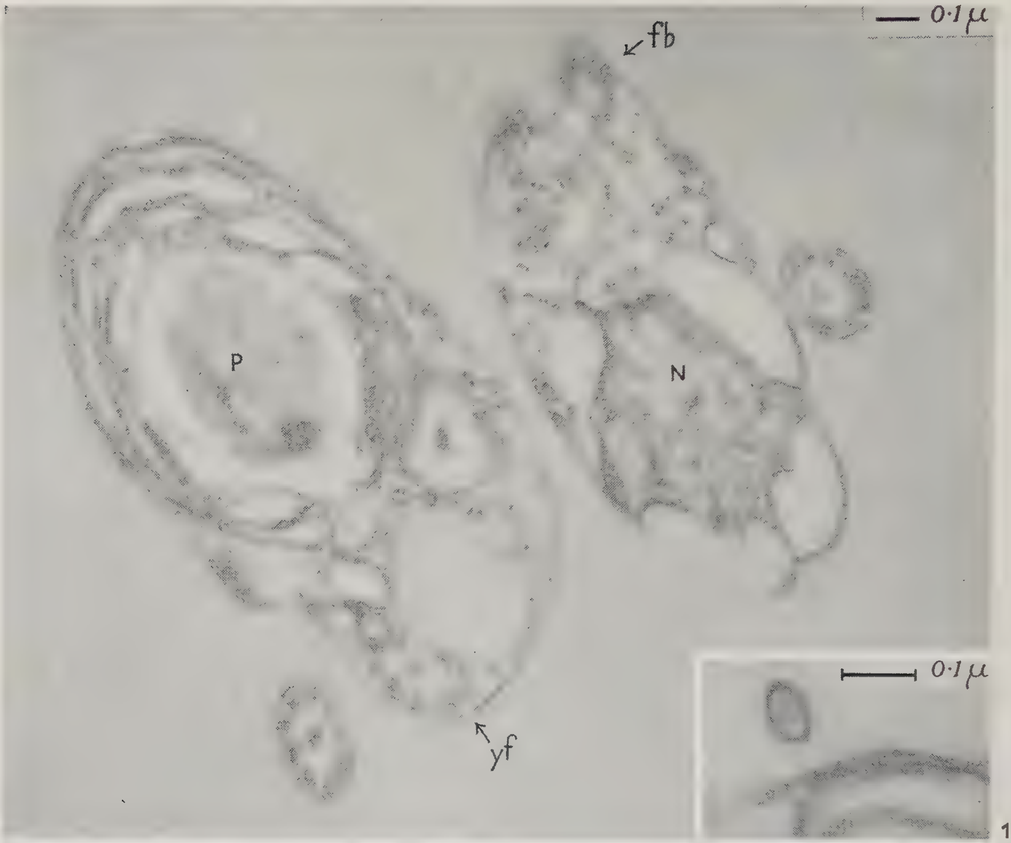
Fig. 14. Two cells in transverse section showing the different parts of the flagellum within the cytoplasm; *fb* (right-hand cell) a basal body in TS; *yf* (left-hand cell) axis of a young flagellum which has not yet emerged from the cytoplasm; nucleus (*N*), plastid with pyrenoid (*P*). For further explanation see p. 329. Micrograph RS 445 × *c.* 55,000.

Fig. 15. TS of a flagellar tip showing contents; for further description see text p. 323. Micrograph MS 298 × 100,000.

Fig. 16. TS through a cell somewhat flattened by a knife, showing details of the mitochondrion (*m*), flagellar axis, and paired lamellae in the plastid (*P*); arrows point to places where the triple structure of the surface membrane is resolved. Micrograph MS 242, × 55,000.

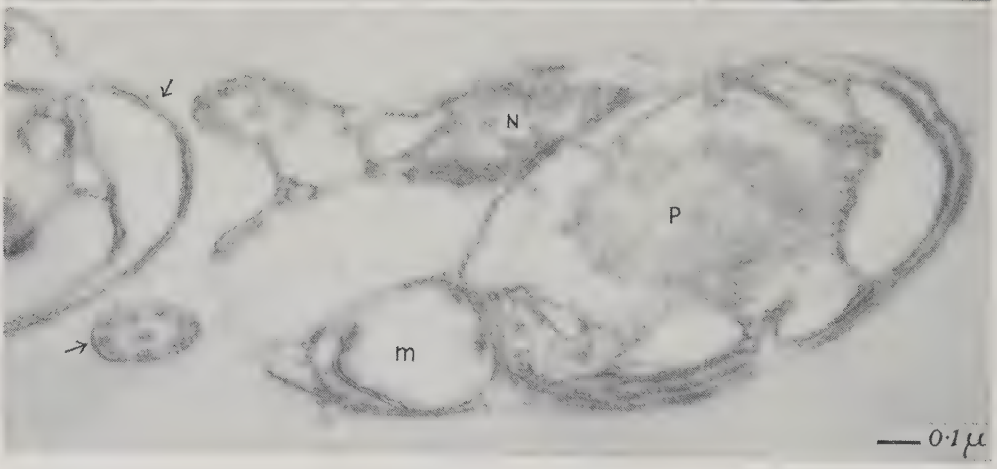

1μ
N
ps
ps
P
m
a
13a
b
13b
N
m
c
13c
2.
d
e
12e

(*Facing p.* 324)


fb
0·1μ
P
N
yf
0·1μ

14

15



16

The plastid

This is by far the largest organ in the cell, and the most conspicuous part of it is the central pyrenoid which is large enough to be traversed by and to dominate almost every type of section except extremely tangential ones, as may be seen in some part of each of Pls. I–X.

There is as yet no chemical knowledge about the nature of the photosynthetic product except that tests for starch with iodine are invariably negative. No suggestions can therefore be made about the chemical nature of the dark central body forming the core of the pyrenoid and which may be expected to be the main food reserve. Outside this central dark area is a narrow lighter layer (well seen in Fig. 12*c*, Pl. V, and elsewhere), outside which is granular material which seems to be part of the normal ground substance between the outer lamellae and which, in small bands, can be encountered at all levels out to the plastid surface (see especially Fig. 5, Pl. II). The plastid surface itself is bounded by a membrane (Fig. 5 and elsewhere) and between this and the pyrenoid are a series of curved membranes joined in pairs at their edges (see especially Figs. 11, 16, 23, etc.). The enclosed spaces within each membrane pair are often distorted into lenticular blisters by the action of the fixative (e.g. Fig. 12 and elsewhere), though in more fortunately fixed specimens the array of membranes lie more evenly parallel to each other (Fig. 11, Pl. IV) in a crescentic path. They do not, however, extend without interruption over the entire circumference of the plastid, some points of interruption being related to the presence of small vesicles of unknown nature (Fig. 11); other points of interruption, which are not detectably so related are also visible in Fig. 11 and elsewhere (see for example Fig. 23, Pl. IX).

This arrangement of lamellae, which is difficult to describe verbally, but which will perhaps become sufficiently clear by further scrutiny of Pls. II, IV, V and VI, is quite unlike that of the Chrysophyceae for which preliminary reports from our own experience are available for *Synura* (see Manton, 1955) and for *Chrysochromulina* (see Parke, Manton & Clarke, 1958, 1959). It is, however, not unlike that recently described for *Chlamydomonas* by Sager & Palade (1957), allowance being made for the difference of size. This was therefore the first reason to suspect that this organism had been wrongly placed.

The mitochondrion

A second reason for suspecting a classificatory error was the fine structure of the single mitochondrion which is always to be found as a sausage-shaped organelle (round in cross-section) in close contact with both plastid and nucleus and not far removed from the base of the flagellum. It may be seen, and is labelled, in Fig. 7, Pl. II, Fig. 11, Pl. IV, both series on Pl. V, and elsewhere. The normal mitochondrial structure in all members of the Chrysophyceae, Phaeophyceae, and Xanthophyceae investigated so far[1] shows an array of closely crowded tubular villi extending from the inside of the mitochondrial wall into the cavity and corresponding to the 'cristae' of most animal mitochondria (Palade, 1952, *et seq.*). In *Chlamydomonas*, however the mitochondria are described (Sager & Palade, 1957) as having a few, widely separated flattened cristae which scarcely interrupt the central lumen. Had this description not appeared as the present enquiry was being carried out we might have been in some doubt as to the true identity of our organelle. As it is the figures cited above, especially the two series of sections contained in Pl. V, and more highly magnified single views such as that of Fig. 16, Pl. VI, leave no doubt that this organ is not only a mitochondrion but a mitochondrion of the chlorophycean but not chrysophycean type.

Peculiar features are the facts that the organelle is single and that the cristae are often curved. The first observation will be discussed again in the section on cell division (p. 327). Curvature of the mitochondrial cristae can be seen best in Fig. 13*a–c*, Pl. V, and in Fig. 16, Pl. VI.

Other cytoplasmic components

Near to the base of the flagellum (Fig. 6, Pl. II) there is a fat body, which once seen can be picked up in other planes of section, e.g. Figs. 12*c–e*. Equally close to the base of the flagellum (Fig. 4, Pl. 1, Fig. 7, Pl. II, etc.) is a compact group of small vesicles which probably represents the equivalent of a golgi area (*x–x*, Fig. 7). There are in addition a few other vesicles of various sizes which are not of this nature. Some good examples are contained in various parts of the cytoplasm not closely associated either with the flagellum or with the nucleus (*v* in Fig. 11, Pl. IV) which seem unlikely to be either artifacts or other organelles.

Lastly there are granules. The amount of granular cytoplasm is very restricted. The large organelles (nucleus, plastid, mitochondrion) are so near to the cell surface on their outer sides that there is little except the body membrane to cover them. Only the angular spaces between these organs, and especially between them and the flagellar base, contain granular cytoplasm in an appreciable amount, together with vesicles of various sizes. This may be examined to advantage in Fig. 7, Pl. II, and Fig. 14, Pl. VI.

Finally the body membrane already discussed in a preliminary way in connexion with the flagellum can be demonstrated independently in Fig. 16, Pl. VI (arrows) as a three-layered structure identical with that of the membrane covering the surface of the flagellum.

---

[1] The literature on this subject is rapidly becoming too voluminous to quote in full but sample micrographs recording some of our own experience of the fine structure of mitochondria in various groups will be found in: Phaeophyceae *Fucus* (Manton & Clarke, 1956), *Scytosiphon* (Manton, 1956); Xanthophyceae *Vaucheria* (Greenwood, Manton & Clarke, 1957, Greenwood, 1959); Chrysophyceae *Chrysochromulina* (Parke, Manton & Clarke, 1958, 1959).

DIVISION STAGES

No attempt has yet been made to study nuclear division in this organism, and no stages of it other than the final stage of a binucleate cell not yet cleaved into two (Fig. 18, Pl. VII) have yet been identified. Several other manifestations of cell division are however conspicuous and, since these add very greatly to our knowledge of certain structural aspects of the other organs, it is important to include at least some of them.

Several stages in the cleavage of both plastid and mitochondrion are represented in Pls. VIII–X. The plastid seems to divide first and the cleavage passes right through the pyrenoid, starting apparently as an intucking of the plastid membrane on the side towards the nucleus. Examples of completely divided plastids are contained in Fig. 20, Pl. VIII and Fig. 26, Pl. X, an important detail of the latter being the demonstration, so far the best available for this organism, that the plastid membrane is probably compound (arrows at base). Incomplete stages are contained in Figs. 22–25, Pls. IX and X. Before scrutinizing these it will perhaps be helpful to notice that whereas Figs. 24 and 25 represent two different cells cut in planes approximately at right angles, Figs. 22*a* and 22*b* are different sections of the same cell, Fig. 22*a* being nearer to the cell surface than 22*b*. Finally Fig. 23, though at an uncertain magnification owing to an uncorrectable mistake in the record made at the time the micrograph was taken, is included for the completeness with which the very large plastid shows numerous areas of lamellar junction (marked by arrows) and an incipient cleavage furrow.

Three stages of cleavage of the mitochondrion are represented by Figs. 22, 26 and 21 (long arrow), the sequence being in the order of citation of the figures. As cleavage of the plastid is progressing the single mitochondrion seems to become partly drawn into the plastid cleavage furrow (Fig. 22*a*); it then becomes bent (Fig. 26) into a U shape, finally a depression resembling a cleavage furrow appears on the concave side (Fig. 21*b*), by which time plastid cleavage is complete and the two halves have begun to separate. Further details regarding the final separation of the mitochondrion halves have not been traced.

Observations on the mode of origin of a new flagellum are much more difficult to make and only a few stages have so far been detected, in most cases by accidental inclusion in fields recorded for other purposes, since it is almost impossible to detect them directly with the eye on the fluorescent screen of the microscope. However, a new flagellum undoubtedly forms close to an old one and it is sometimes possible, in dividing cells, to pass through the two basal bodies side by side (Fig. 19, Pl. VII). It is therefore likely that the new basal body from which the new flagellum will grow out must be formed in some structural relation to the old basal body, but what this relation is has not yet been elucidated. However, one fortunate series of sections which happened to lie close to the field of Fig. 14 is reproduced in Figs. 17*a–f*, Pl. VII, to illustrate a very young flagellum in an early stage of growing out. It is slightly tilted and the basal body is best seen in the lowermost of the six sections (Fig. 17*f*). The central strands passing out into a very short hair point are contained in Fig. 17*d*, the tip of the short hair point itself being crossed by the path of an independent long hair point which can be seen passing obliquely through all the sections. The 9 strands of the peripheral ring are distributed among all six sections, though it should be noted that these strands are still entirely within the body membrane of the parent cell.

This observation on an undoubtedly young specimen is important for providing a clue to some of the differences in external length and in the position of the basal body relative to the surface, to which reference has been made on previous pages, especially in relation to the longitudinal sections included on Pls. II and III. It is obvious that after a new flagellum has been laid down and

---

Explanation of Plates VII–X

Chromulina pusilla Butcher

VII

Fig. 17*a–f*. Series of six adjacent sections passing longitudinally through a young flagellum not yet emerged from the subtending cell. For further explanation see text. Micrographs RS 440, 441, 442, 443, 444, 445, × *c*. 30,000.

Fig. 18. Sections through a dividing cell showing two nuclei. Micrograph H 2278 × 30,000.

Fig. 19. Section through a dividing cell with two ciliary bases side by side (arrows). Micrograph MS 35, × *c*. 30,000.

VIII

Fig. 20. Group of cells one of which shows a divided chloroplast. Micrograph MS 44, × *c*. 25,000.

Fig. 21*a* and *b*. Two successive sections through a field of three cells the lowest of which contains a dividing mitochondrion (21*b*) and a divided plastid (21*a* and *b*). Micrographs RS 416 and 417, × *c*. 25,000.

IX

Fig. 22*a* and *b*. Two sections (not immediately adjacent) through a dividing cell showing early stages of division of the mitochondrion (22*a*) and plastid (22*b*). Micrographs H 2113 and 2121, × 30,000.

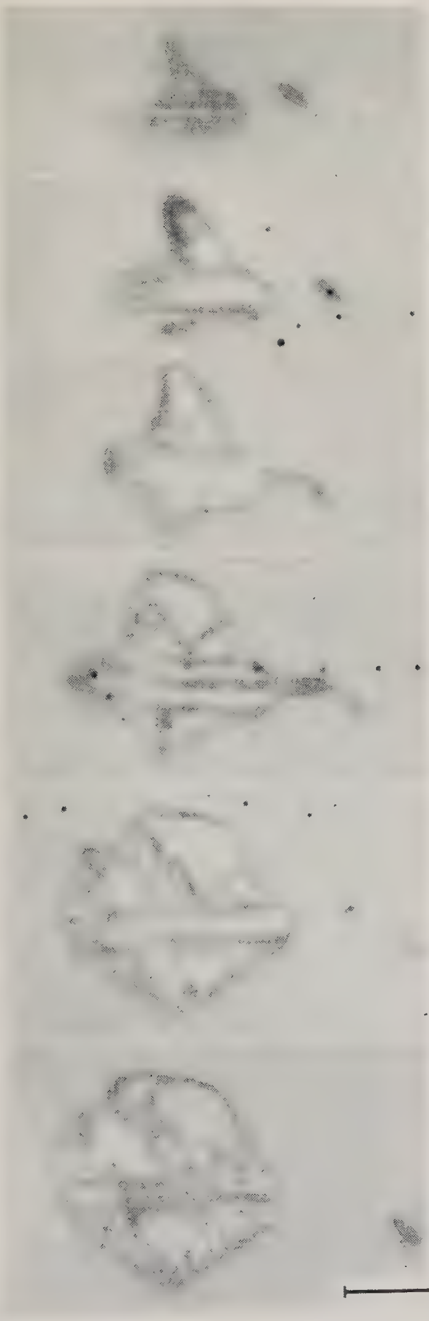
Fig. 23. A giant cell (probably a 'double-division') to show details of an early stage of fission of the plastid, arrows indicate places of fusion of lamellae. Micrograph H 2123, magnification uncertain but probably between 40,000 and 50,000.

X

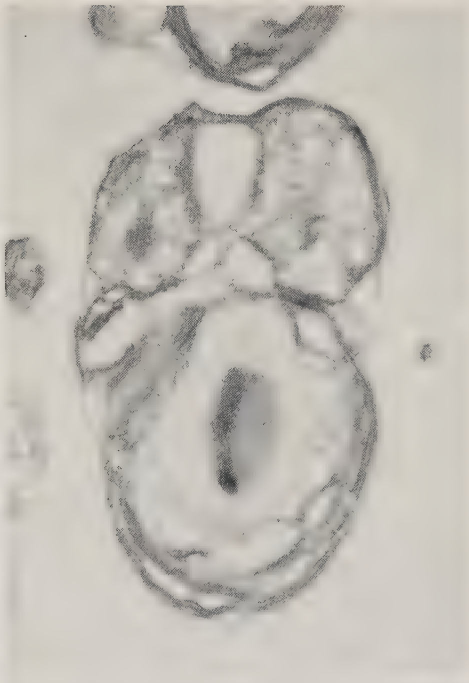
Fig. 24. A cell cut transversely to the plane of flattening showing a dividing plastid in an intermediate condition. Micrograph H 2126 × c. 30,000.

Fig. 25. A cell at approximately the same stage as that of Fig. 24 cut in a place at right angles to that of Fig. 24. This section includes a nucleus above the dividing plastid. Micrograph H 2115, × *c*. 30,000.

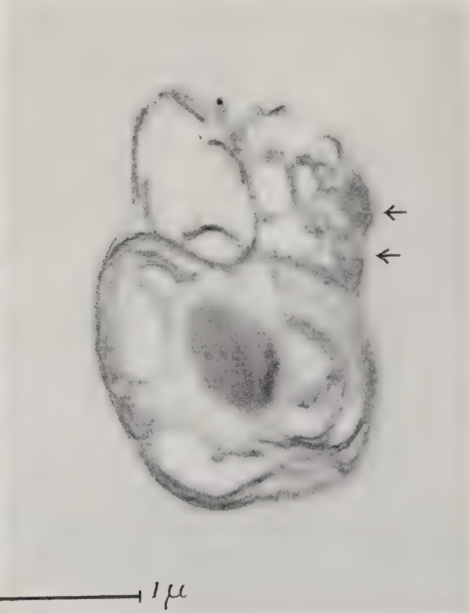
Fig. 26. A cell with a divided plastid and a dividing mitochondrion (*m*) more highly magnified to show among other details the multilayered structure of the plastid membrane (arrows). Micrograph H 2298, × 80,000.



17f

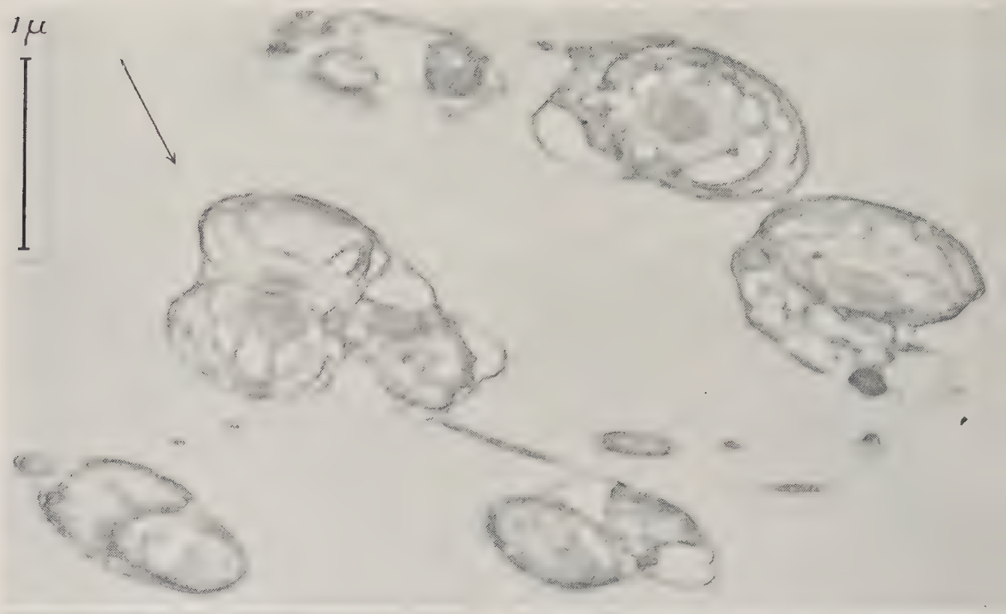


18

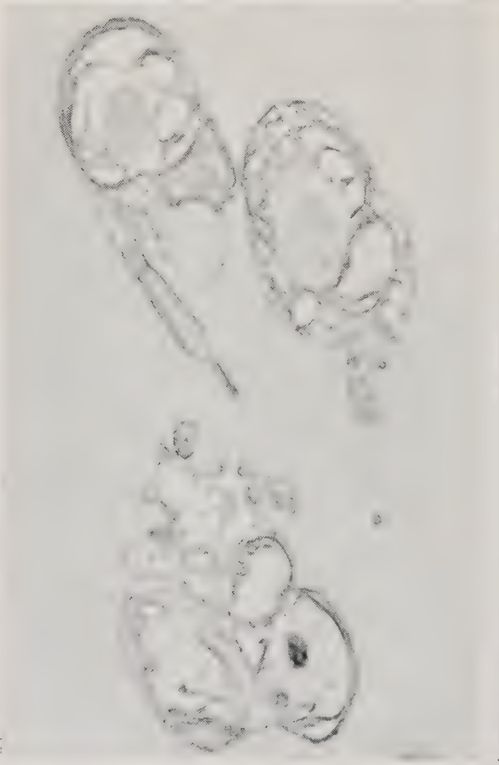


19

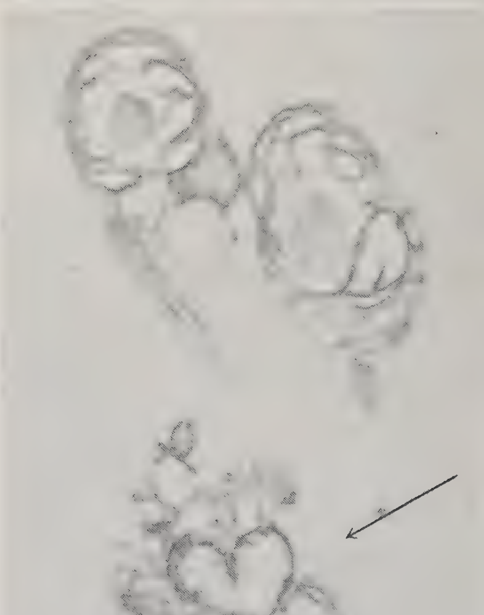
(*Facing p.* 328)



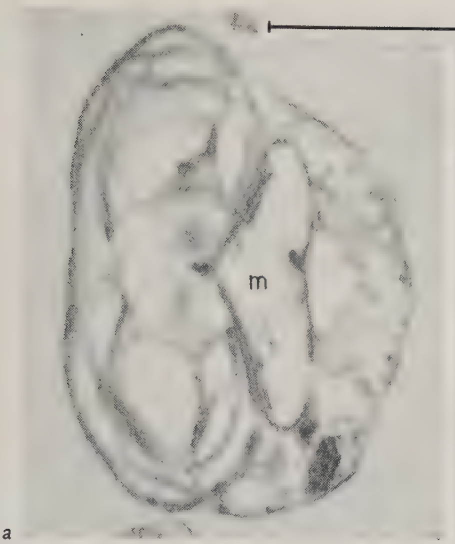
20



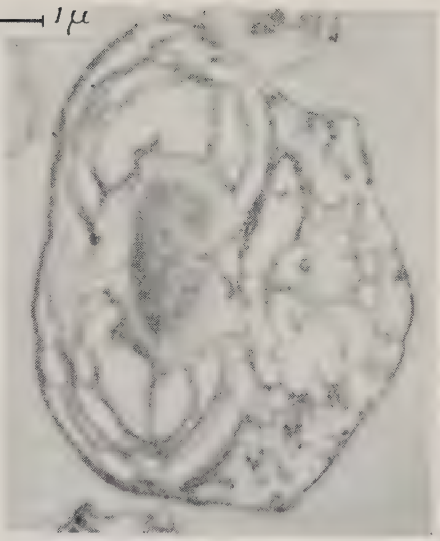
21a



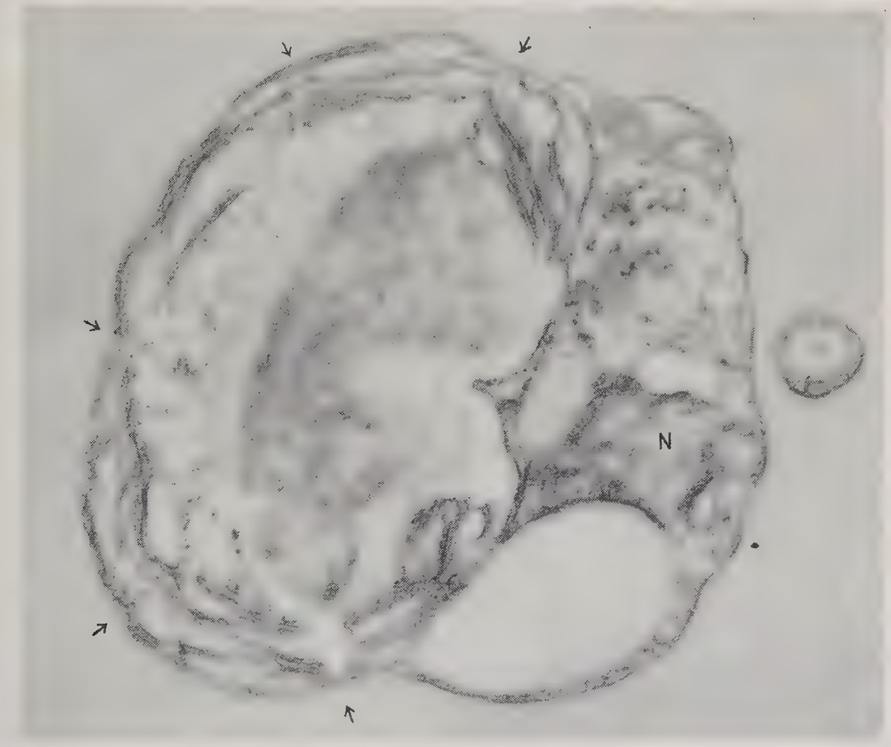
2



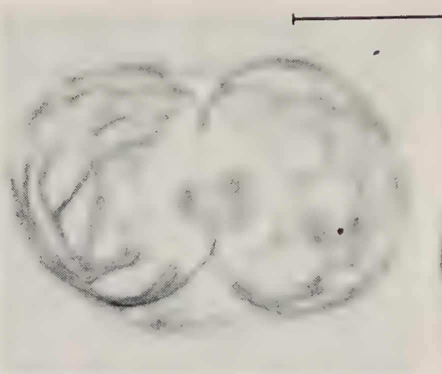
22*a*



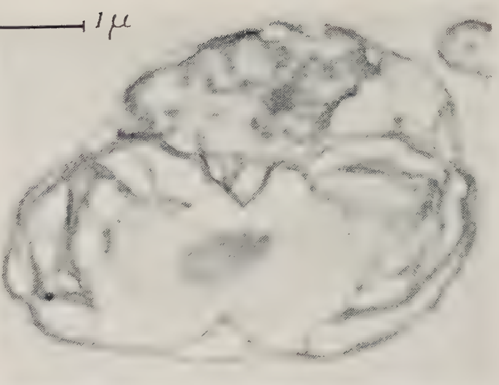
22*b*



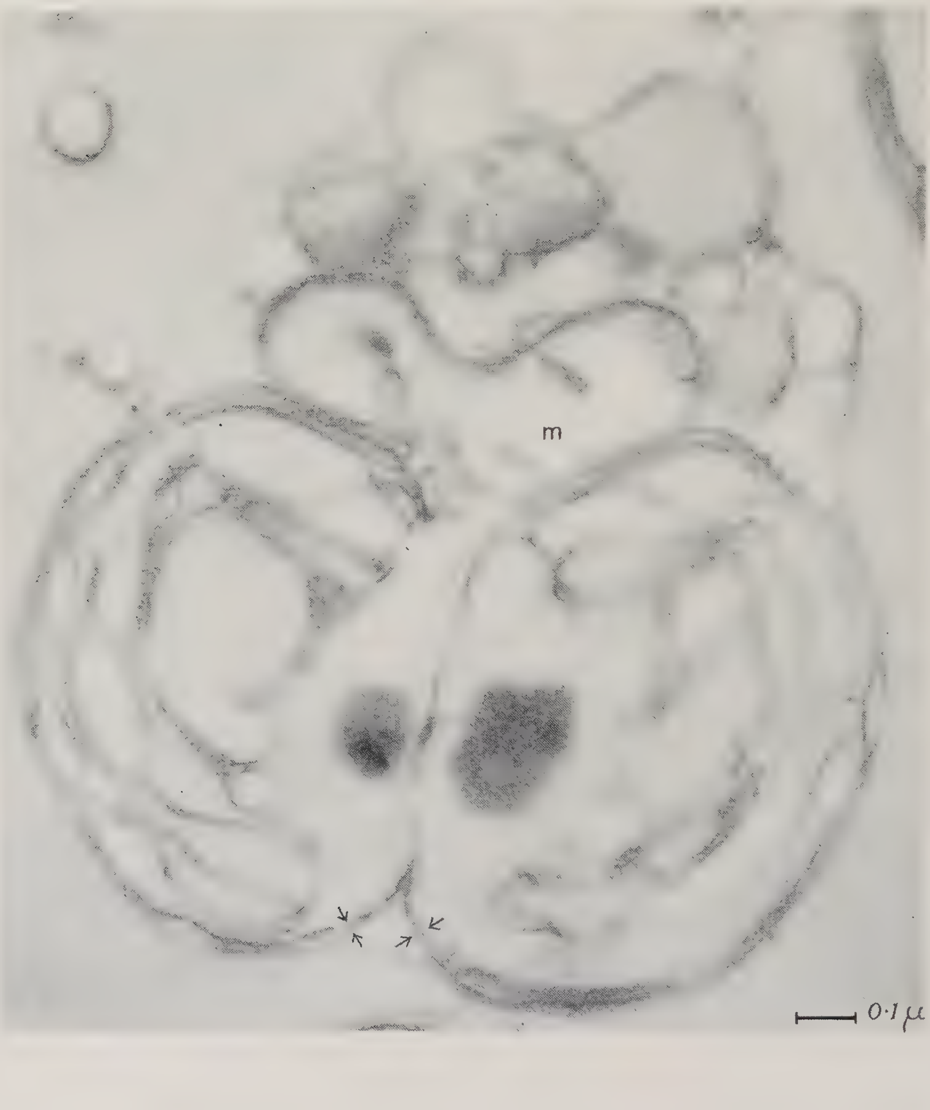
23



24



25



26

has elongated within the cell (not necessarily to its full size) there must at some point be an active movement of its basal body from a deep-seated position out to the surface, which is not true growth although externally it may resemble it. This movement could be very rapid, but until it has been completed a transverse section of a cell at a suitable level will show an internal flagellar axis without a separate membrane as in the left-hand cell of Fig. 14 (*yf*), Pl. VI, while a similar section taken further out can show a partially detached flagellum as in Fig. 16, Pl. VI. Comparison of both these figures with the longitudinal view of Fig. 10, Pl. III, will at once make this matter clear, and it will also be apparent by way of contrast that for any cell which had reached the condition of Fig. 8, Pl. III, a transverse section at no matter what level could at most include the flagellar basal body (*fb* in the right-hand cell of Fig. 14) within the subtending cytoplasm but no part of the flagellar axis.

DISCUSSION

Ignoring for a moment the taxonomic implications of the facts presented there are several points in the structure of this curious little organism which are of potential interest to students of fine structure. The first of these is the extreme numerical reduction of all the component parts in correlation with the greatly reduced overall dimensions of the cell. By the time that a cell is restricted to one nucleus, one plastid, one mitochondrion, one little group of golgi vesicles, one fat body, one flagellum and a very small amount of residual cytoplasm, it seems doubtful whether any further reduction would be possible without involving the fundamental structure of the organelles themselves. This has however not yet occurred in this species. Qualitatively all the organelles even the greatly abbreviated flagellum, are essentially of the same type as those of other algal cells, allowance being made for minor differences in relative dimensions of which those shown by the flagellum are the most extreme. Even here, however, the flagellum is only exceptional in the extreme disproportion between the flagellum proper (the thickened base) and its hair-point (the whiplash distal part). Hair-points are nevertheless in themselves common enough though usually short (see Manton, Clarke & Greenwood, 1955) but even long hair-points can sometimes be found, occasionally as an abnormality, as in *Fucus* (see Manton & Clarke, 1951), or even sometimes perhaps as a permanent feature (see Petersen, Caram & Hansen, 1958, on *Chordaria*). In none of these other organisms has it been shown that the hair-point contains a prolongation of the central strands, so that strict homology cannot yet be claimed. It is nevertheless at least possible that the exceptional condition about our present species might be not so much the permanent presence of a long-hair point as the fact that the flagellum proper is so short that it could be mistaken for a swollen base to the other organ. When this 1 μ long basal part is recognized for what it is the transverse dimension is not fundamentally different from that of any other plant flagellum for which the diameter in sections is known.[1]

The reduction of the mitochondria to one only is a highly interesting feature for which we have no other examples at present. The observations made on cleavage, both of the plastid and the mitochondrion, suggest that in this organism both are self-propagating organelles which are not, and probably cannot be, reformed from any other cell component. For the mitochondria this has long been suspected, though in ordinary circumstances it is difficult to prove, since in a cell with many mitochondria it is usually impossible merely from the shapes to distinguish with certainty between division stages and coalescence. In this case the latter explanation of the shapes encountered seems excluded and we can only logically interpret the stages seen as reproduction by division.

For the plastid this is well known in many algae since green chromatophores are commonly quite large enough to be studied alive in considerable detail. In the lower algae of all colours other than blue-green it is a commonplace that mature plastids often divide without ever being resynthesized *de novo*. Nevertheless in many algae and in all or almost all higher plants the actual plastid is commonly developed not from another mature one but from a colourless primordium which alone has the capacity for division. This condition is, however, unlikely to be primitive and one may believe it not yet to have been reached by algal cells at the evolutionary level of our present flagellate.

The growth of the flagellum is a matter of considerable interest since this is a subject which, in plants, has not yet been effectively studied. Much would be learnt if it were possible to detect the first beginnings of a new flagellum. The much more limited observations made here of growth from a base inside the cell (the series of Fig. 17, Pl. VII) are nevertheless of importance in showing among other things that the flagellar membrane is not merely like the body membrane and continuous with it but is actually a part of the body surface which has been lifted up from below by growth of the flagellum itself. This could not have been deduced with certainty by observation of the mature structure, and it is a fact which will be of considerable comparative interest as knowledge of other organisms increases.

---

[1] Exact measurements of the diameter of cilia and flagella have not been published as such nor are they easy to obtain with any accuracy owing to the distortions inseparable from the processes involved in their study. Nevertheless a rough estimate can be obtained by comparing the published micrographs of the best fixed specimens for which, when expressed as vulgar fractions to avoid falsely suggesting greater accuracy than is justified, we obtain the following:

Sphagnum (Manton, 1957)	1/4μ
Fucus (Manton & Clarke, 1956)	1/5μ
Scytosiphon (Manton, 1956)	
Chrysochromulina (Parke, Manton & Clarke, 1958)	
Vaucheria (Greenwood, Manton & Clarke, 1956)	1/6μ
'*Chromulina pusilla*'	1/7μ

With regard to the other aspect of the investigation, namely, the taxonomic implications, it is obvious that we have here unintentionally entered a highly specialized field, of active concern to students of plankton but one which can only be effectively dealt with by an expert professional algologist, which we do not claim to be. Nevertheless the general principles are clear enough, for in addition to our own previous work on other members of the Chrysophyceae and related groups, there is a recent electron microscopical study on *Chromulina psammobia* by Fauré-Frémiet and Rouiller (1957) which proves beyond doubt that the anomalous characteristics of '*Chromulina pusilla*' are not shared by other species more properly included in that group, and that, in addition, our species lacks several characters such as the hairy appendages on a forwardly directed flagellum which are known to exist in other species of the genus.

A conclusive piece of further evidence can moreover be quoted. By cooperation between Dr M. Parke of Plymouth and Dr G. Y. Kennedy of Sheffield a large amount of material was cultured and subjected to qualitative pigment analysis. The findings (Kennedy, personal communication dated 15 November 1957) were as follows: 'present: chlorophyll *a*, chlorophyll *b*, no trace of any other chlorophyll; α and β carotene, lutein; no other pigments'. The decisive factor in this list is of course chlorophyll *b* which is entirely absent from all known representatives of the Chrysophyceae but which is virtually diagnostic of Chlorophyceae and near relatives. Its presence is the final and unequivocal proof that this organism must be removed out of the genus *Chromulina* and out of the class Chrysophyceae.

Whether, when this is done, it can properly be included within any existing genus of Chlorophyceae or whether indeed an entirely new group of non-starch-producing flagellates will be required to contain it are matters on which it would be wrong at this stage to express further opinions. Dr M. Parke is actively engaged in exploring the various alternatives and may be expected to publish her findings shortly. In the meantime it may be helpful to enumerate the taxonomically significant characters which the electron microscope has revealed and which will need to be covered by any generic diagnosis which may eventually be selected. An emended description, not including any additional facts which may still be ascertainable with the light microscope, is therefore as follows:

Unicellular pigmented flagellate, of average size *c.* $1 \times 1{\cdot}5\,\mu$, possessing chlorophylls *a* and *b*, carotenes α and β, and lutein, but not forming starch; with a single plastid possessing a large central pyrenoid covered by a shell of concentric paired lamellae resembling in a general way those of the Chlorophyceae; with a single mitochondrion possessing a sparse array of flattened cristae resembling those of certain green algae, notably *Chlamydomonas*, and with a laterally attached flagellum *c.* $1\,\mu$ long terminating in a slender hair-point *c.* $3\,\mu$ long, both parts directed backwards during swimming; the body smoothly covered by a membrane but without surface scales or cell wall; multiplication by fission involving the plastid, the mitochondrion and the nucleus with the formation of a new flagellum immediately beside the old one.

SUMMARY

The very small flagellate hitherto known as *Chromulina pusilla* Butcher has been examined morphologically and anatomically with the electron microscope. The cell is found to contain a nucleus, plastid, one mitochondrion, one small golgi area, one fat body, a small amount of granular and vesicular cytoplasm and a flagellum less than 1 μ long terminating in an extended hair-point, the whole surface being covered by a three-layered membrane. In spite of their small size all parts are structurally normal. The anatomical and biochemical evidence indicates that this flagellate has been placed in the wrong group. The details given include a few observations on division stages of the plastid and mitochondrion and on the growth of the flagellum. An emended diagnosis is inserted as a guide to the ultimate reclassing and renaming of the organism.

REFERENCES

BUTCHER, R. W., 1952. Contributions to our knowledge of the smaller marine algae. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 175–91.

FAURÉ-FRÉMIET, E. & ROUILLER, C., 1957. Le flagelle interne d'une Chrysomonadale: *Chromulina psammobia. C.R. Acad. Sci., Paris,* T. 244, pp. 2655–7.

GREENWOOD, A. D., MANTON, I. & CLARKE, B., 1957. Observations on the structure of the zoospores of *Vaucheria. J. exp. Bot.*, Vol. 8, pp. 71–86.

GREENWOOD, A. D., 1959. Observations on the structure of the zoospores of *Vaucheria.* II. *J. exp. Bot.*, Vol. 10, pp. 55–68.

KENNEDY, G. J. & NICOL, J. A. C., 1959. The pigments of *Chaetopterus. Proc. roy. Soc.* B (in the press).

KNIGHT-JONES, E. W. & WALNE, P. R., 1951. *Chromulina pusilla* Butcher, a dominant member of the ultraplankton. *Nature, Lond.*, Vol. 167, p. 445.

KNIGHT-JONES, E. W., 1951. Preliminary studies of nanoplankton and ultraplankton systematics and abundance by a quantitative culture method. *J. Cons. int. Explor. Mer,* Vol. 17, pp. 140–55.

MANTON, I., 1955. Observations with the electron microscope on *Synura caroliniana* Whitford. *Proc. Leeds phil. lit. Soc.* vol. 6, pp. 306–316.

—— 1956. Observations with the electron microscope on the internal structure of the zoospore of a brown alga. *J. exp. Bot.*, Vol. 8, pp. 294–303.

—— 1957. Observations with the electron microscope on the cell structure of the antheridium and spermatozoid of *Sphagnum. J. exp. Bot.*, Vol. 8, pp. 382–400.

MANTON, I. & CLARKE, B., 1951. An electron microscope study of the spermatozoid of *Fucus serratus. Ann. Bot.*, N.S., Vol. 15, pp. 461–71.

—— —— 1956. Observations with the electron microscope on the internal structure of the spermatozoid of *Fucus. J. exp. Bot.*, Vol. 7, pp. 416–32.

MANTON, I., CLARKE, B. & GREENWOOD, A. D., 1955. Observations with the electron microscope on biciliate and quadriciliate zoospores in green Algae. *J. exp. Bot.*, Vol. 6, pp. 126–8.

PALADE, G., 1952. The fine structure of mitochondria. *Anat. Rec.*, Vol. 114, pp. 427–52.

PARKE, M., MANTON, I. & CLARKE, B., 1958. Studies on marine flagellates. IV. Morphology and microanatomy of a new species of *Chrysochromulina. J. mar. biol. Ass. U.K.*, vol. 37, pp. 209–228.

PARKE, M. MANTON, I. & CLARKE, B., 1959. Studies on marine flagellates. V. Morphology and microanatomy of *Chrysochromulina strobilus* sp. nov. *J. mar. biol. Ass. U.K.*, Vol. 38,pp. 169–188.

PETERSEN, J. B., CARAM, B. & HANSEN, J. B., 1958. Observations sur les zoïdes du *Chordaria flagelliformis* au microscope électronique. *Bot. Tidsskr.*, Bd. 54, pp. 57–60.

SAGER, R. & PALADE, G. E., 1957. Structure and development of the chloroplast in *Chlamydomonas*. I. The normal green cell. *J. biophys. biochem. Cytol.*, Vol. 3, pp. 463–87.

J. Mar. biol. Ass. U.K. (1959) 38, 335–350
Printed in Great Britain

THE EARLY GROWTH STAGES AND ADULT STRUCTURE OF THE LOPHOPHORE OF *MACANDREVIA CRANIUM* (MÜLLER) (BRACHIOPODA, DALLINIDAE)

By D. ATKINS, D.Sc.

From the Plymouth Laboratory

(Text-figs. 1–11)

Although it has been stated (Thomson, 1927, p. 234; Elliott, 1953, p. 264) that the descending branches of the loop in dallinids, so far as is known, grow from the crura only, yet figures supporting this statement have not so far been published, not even by Beecher (1895) who described the very early stages of lophophore and loop in *Dallinella* (= *Terebratalia*) *obsoleta* (Dall). Tiny specimens of *Macandrevia cranium* dredged by R. V. 'Sarsia' have now made it possible for such figures to be published. It is a point of some importance as it is one of the characters given by Thomson (1927, p. 234) as distinguishing the Dallininae from the Mühlfeldtiinae (= Megerliinae Muir-Wood, 1955) and the Magellaniinae (= Terebratellinae, see Muir-Wood, 1955), for in the two latter groups the descending branches of the loop grow from both crura and septum to unite in the middle. At the time Thomson was writing, young growth stages of the loop of a member of the Laqueinae were not known. Since then, Konjoukova (1948, 1957) has studied the development of *Laqueus californicus*—which she placed in the Dallininae—and according to her the descending branches on reaching the septum join with it. She does not, however, figure any intermediate stages between absence of descending branches and complete ones. In 1941 Laqueinae was raised to family rank by Yabe and Hatai (see Muir-Wood, 1955, p. 93).

Palaeontologists have given the growth stages of the loop various names derived from different genera. Certain of the genera are fossil, and although familiar to palaeontologists, are unlikely to be so to many zoologists. Moreover, these terms are not fixed, several of them having been changed by successive authors, as may be seen from the table given by Thomson (1927, p. 232); since that date a further change has been made by Elliott (1953, p. 264), although not followed by Konjoukova in her 1957 monograph. It has therefore been decided to avoid them, relying on the figures to make the stages clear.

All figures have been drawn with the aid of a camera lucida. Mantle setae have been omitted from the figures as they cover much space: at a shell length of 1·4 mm the anterior setae reach a length of about 0·8 mm.

Macandrevia cranium lacks the two carmine pigment spots, which have been found near the preoesophageal ganglion in certain other brachiopods.

THE EARLY GROWTH STAGES OF THE LOPHOPHORE AND LOOP

The fullest series of growth stages of the loop—without the lophophore—of *Macandrevia cranium* so far described and figured is that of Friele (1877) from a shell length of a little less than 3 mm to the adult. He remarked that at the former size 'a coherent apophysary system' first occurs. He had younger stages, but in a dried condition, and did not figure their brachial support. Elliott (1948) described and figured, from preserved material, the development of the lophophore and loop from a shell length of 5 mm to the adult.

Fine shell gravel from near La Chapelle Bank provided numbers of young *M. cranium* of shell length 1·0 mm onwards. Of two hauls, the first, that taken on 19 June 1956 at position 47° 37′ N., 7° 16′ W. and depth of 90–100 fathoms, was the richer of the two in tiny brachiopods, *Terebratulina retusa* and *Gryphus vitreus* occurring in addition to *Macandrevia cranium*. It was preserved on board and thus the lophophores were unfortunately in a contracted state. The second haul of 20 April 1958 from position 47° 35′ N., 7° 13′ W. at a depth of 105–110 fathoms was brought back under circulation and some young living *M. cranium* were found.

Stages of the lophophore at a shell length of 1·0–5·3 mm will be described and figured so as to join with and somewhat overlap Elliott's (1948) series. The stage of development of the lophophore to shell length varies somewhat in the La Chapelle Bank *Macandrevia*, so that length can only be taken as approximate for the stage under consideration; however, the La Chapelle Bank material shows earlier development of the loop than the specimens from Norway used by Friele (1877) and Elliott (1948).

The earliest stages were found in the preserved shell gravel and show the lophophore in the contracted state (Fig. 1).

The lophophore is trocholophous to the time when eleven pairs of filaments have developed—the last pair being short—with in addition a minute asymmetrical bud. The base of the lophophore, or lophophoral ridge, is a closed circle (Fig. 1 A), as described by Beecher (1895, p. 393) in *Dallinella* (= *Terebratalia*) *obsoleta* (see, however, Williams, 1956, pp. 260–1). The more recently formed filaments show a marked difference in length, indicating that the appearance of new ones is not as rapid as in later stages. The first few filaments to be formed arise from the body behind the mouth; then it would seem that the lophophoral ridge extends on to the brachial mantle. The mouth is a small opening lying in front of the bases of the first formed pair of filaments, as described by me (Atkins, 1959) in *Platidia* and by Beecher (1895) in *Dallinella obsoleta*. In front of the mouth is a lobe which elongates laterally —running concentric to the bases of the filaments—to form the lip of the food groove or brachial fold. At the eleven pairs of filament stage the lip reaches to about the base of the fifth or sixth filament on each side. The length of the shell at this stage is approximately 1·0–1·2 mm. This mode of growth of the lip has been previously described in *Platidia* (see Atkins, 1959) and is contrary to that described by Percival (1944) and by Williams (1956, p. 260).

By the time twelve pairs of distinct filaments are present the lophophoral ridge is interrupted in the mid-line anteriorly and very slightly indented, there now being two distinct, but closely contiguous, growth regions (Fig. 1 B, C).

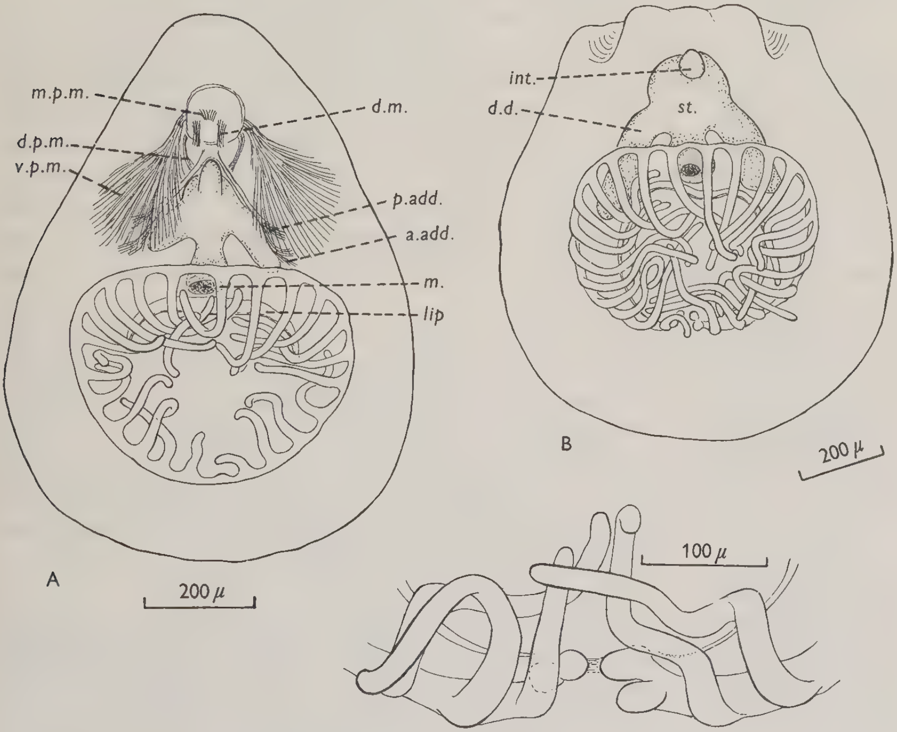


Fig. 1. *Macandrevia cranium*. A, Entire animal of shell length 1·0 mm viewed ventrally: lophophore trocholophous. B, Brachial valve of specimen of shell length 1·35 mm with very early schizolophe: muscles omitted. In both A and B the lip of the food groove (*lip*) is turned outwards exposing the mouth (*m.*). C, Growing region of the lophophore of a specimen of shell length 1·25 mm; the lip of the food groove is merely a slight ridge anteriorly. *a.add.*, anterior adductor muscle; *d.d.*, digestive diverticulum; *d.m.*, diductor muscle; *d.p.m.*, dorsal pedicle muscle; *int.*, intestine; *m.p.m.*, median pedicle muscle; *p.add.*, posterior adductor muscle; *st.*, stomach; *v.p.m.*, ventral pedicle muscle. Stained with eosin and drawn while in cedar wood oil.

The filaments are still in a single row and all have ridged frontal surfaces. In addition to the twelve pairs of recognizable filaments are two pairs of minute buds (Fig. 1 B, C) and not the one minute asymmetrical bud as in the trocholophous stage. No crura or septum can be recognized. The growth of the lip of the food groove has caught up, or nearly so, with that of the filaments, and it extends to about the base of the last formed filament on each side. Shell length is now 1·2 –1·4 mm.

From this stage onwards, as the invagination of the lophophore deepens, the rate of growth of new filaments is accelerated, the growing region bearing closely gradated buds (Fig. 2). When twelve to fourteen pairs are present, differentiation into an alternating series of inner filaments, with ridged frontal surfaces, and outer filaments, with grooved frontal surfaces, begins.

It will be seen from a comparison of Figs. 1 B and 2 that the twelfth pair of filaments present in the mid-anterior line at the earlier stage do not remain there, but are displaced laterally by the formation of new filaments as growth proceeds. In the adult the twelve first-formed filaments, to which another one or two pairs with ridged frontal surfaces may be added, come to lie behind the mouth, as may be recognized from their arrangement in a single series.

When the shell has reached a length of about 1·9 mm the position where the septum will arise is indicated by a region where the shell mosaic is finer than over the rest of the valve: crura are present and descending branches have begun to grow anteriorly.

In another specimen of about the same size a small septal boss is just recognizable between the now further separated growth regions. The descending branches of the loop, growing from the crura, reach nearly half way around the base of the lophophore; one is usually more advanced than the other. It seems that the septum appeared earlier with regard to the descending branches in Friele's specimens than in those from La Chapelle Bank, for he records (1877, p. 385) that in a specimen of shell length 2·3 mm, although the 'crura-processes' only had developed, the septum occurred in the form of a lump in the bottom of the valve.

At a shell length of some 2 mm the septum is easily discernible in the anterior invagination of the lophophore (Figs. 2, 3). The descending branches have extended much further anteriorly (Fig. 3), that on the right having almost reached the septum: they finally fuse with it before growth of the hood. As may be seen from Fig. 3, there is no growth from the septum to join with the descending branches growing from the crura. The descending branches are calcareous ribbons vertical to the valve floor, and so appear extremely narrow in dorsal and ventral view; they run slightly above the valve floor.

Fig. 2 A and B of the same lophophore stage, the one drawn living and the other preserved, show the great difference in appearance of the lophophore resulting from preservation without narcotizing (see also Atkins, 1959, p. 128). The individual shown in Fig. 2 B has the septum and descending branches

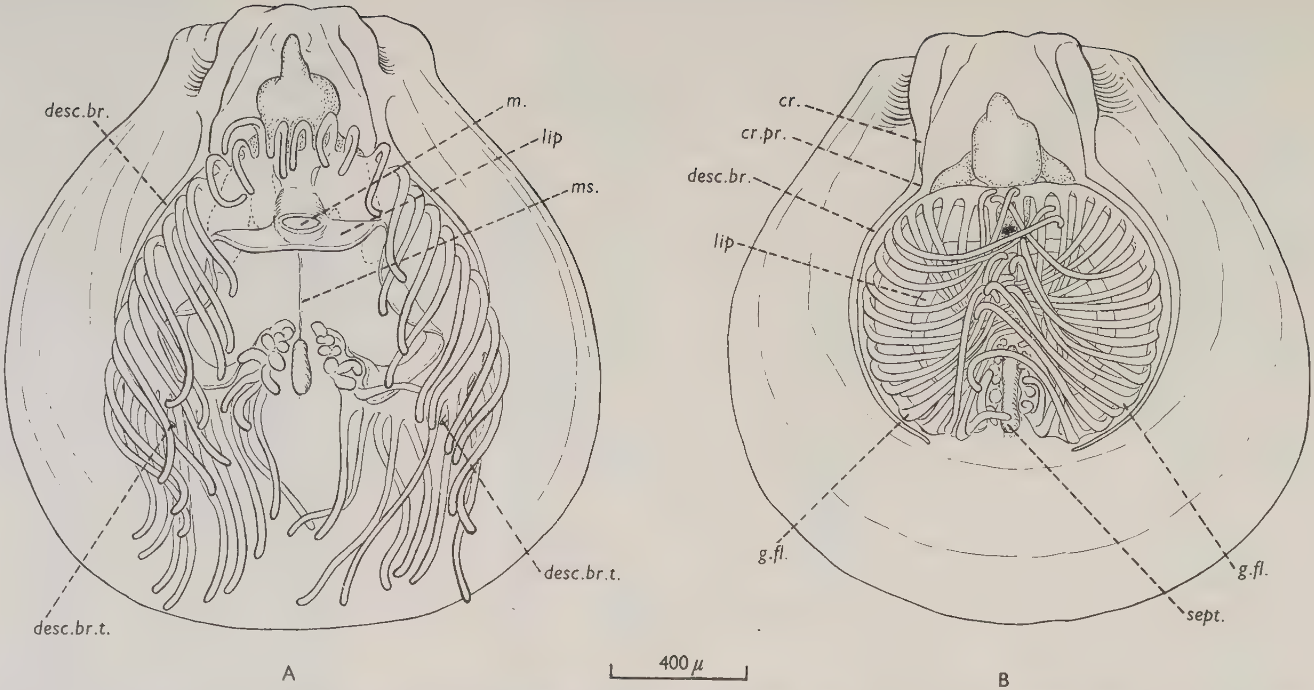


Fig. 2. *M. cranium.* A, Brachial valve, with schizolophe, of specimen of shell length of 2·25 mm, drawn living and fairly well expanded. B, Brachial valve with schizolophe in contracted state, thus exposing the well advanced descending branches of the loop. The septum as yet shows no development of a hood. Specimen of shell length 2·1 mm, drawn preserved without narcotizing. Differentiation into a double series of filaments has begun, the first outer grooved filament on each side is indicated (*g.fl.*). *cr.*, one of the crura; *cr.pr.*, crural process; *desc.br.*, descending branch of the loop; *desc.br.t.*, tip of descending branch; *lip*, lip of the food groove; *m.*, mouth; *ms.*, mesentery; *sept.*, septum.

further developed than that in Fig. 2A, although slightly the smaller of the two.

Transverse sections (Fig. 4) of a specimen of shell length 2·1 mm with schizolophe, shows that in its early stages the lophophore is set low on the brachial mantle. Small brachial canals (*b.c.s.*) are present, and also probably great brachial canals (*b.c.g.*), but owing to their small size at this stage it is impossible to be certain as there is some collapse of their walls. Fig. 4A clearly shows the relation of the mouth (*m.*) to the brachial membrane (*b.m.*) and to the circle of filaments (*fl.*). The descending branches (*desc.br.*) are not confluent with the valve floor, running slightly above it. The section illustrated in Fig. 4C, through the anterior region of the lophophore and through the septum, is near the anterior limit of the descending branches, that on the right alone being present.

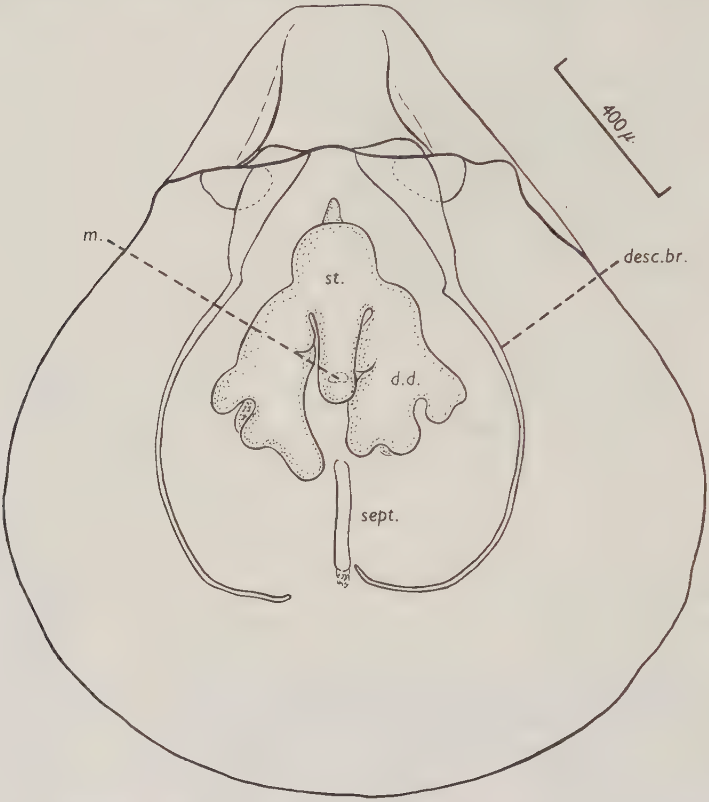


Fig. 3. *M. cranium* of shell length 2·0 mm to show the brachial support, viewed dorsally through the shell: the lophophore is omitted. The descending branch (*desc.br.*) of one side has almost reached the septum (*sept.*). *d.d.*, digestive diverticulum; *m.*, position of the mouth; *st.*, stomach. Stained with eosin and drawn while in cedar wood oil.

As growth of the lophophore proceeds the position of the descending branches shifts inwards. A late schizolophous stage, with lateral arms deflected, at a shell length of 2·2 mm is illustrated (Fig. 5 A). The, as yet widely curved, descending branches have united with the septum, which is slightly grooved ventrally—the grooving precedes formation of the hood—and is split anteriorly. Konjoukova (1957, pp. 63–4) has noted that anterior division of the septum is characteristic of the development of the brachial apparatus of the Dallininae, in which she included *Laqueus*.

In another specimen of about the same size and lophophore stage as that shown in Fig. 5 A the descending branches make an acute angle with the

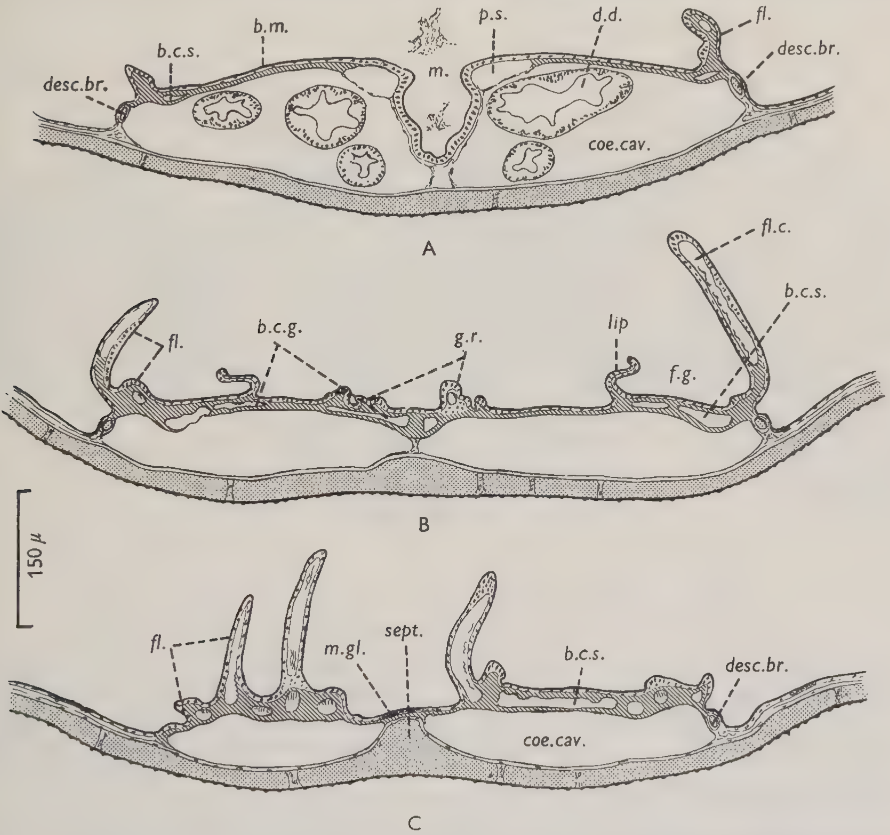


Fig. 4. *M. cranium* of shell length 2·1 mm. Transverse sections through schizolophe to show its relation to the dorsal mantle and shell, (A) through the mouth (*m.*), (B) through the twin growing regions (*g.r.*) and (C) through the anterior region of the lophophore and the septum (*sept.*). *b.c.g.*, great (?) and *b.c.s.*, small brachial canals; *b.m.*, brachial membrane; *coe.cav.*, coelomic cavity; *d.d.*, tubule of digestive diverticulum; *desc.br.*, descending branch of the loop; *f.g.*, food groove; *fl.*, filament and filament base; *fl.c.*, filamentar canal; *lip*, lip of the food groove; *m.gl.*, mucous gland cells; *p.s.*, perioesophageal sinus. Supporting substance diagonally hatched.

septum (Fig. 5 B). This is the youngest stage of the brachial support described by Friele (1877) at a shell length of a little less than 3 mm, nearly agreeing with his fig. 1.

At a somewhat greater shell length, 2·8 mm, the lophophore is still schizolophous, but the ventral, abfrontal, surfaces of the lateral arms have widened. The hood, although narrow is clearly distinguishable. Long, spinous, processes have developed from the anterior end of the septum. This would correspond to Friele's fig. 2. Elliott (1953, p. 269) has noted the spiny nature of many dallinid loops, mature and immature, a feature almost completely lacking in the Terebratellidae.

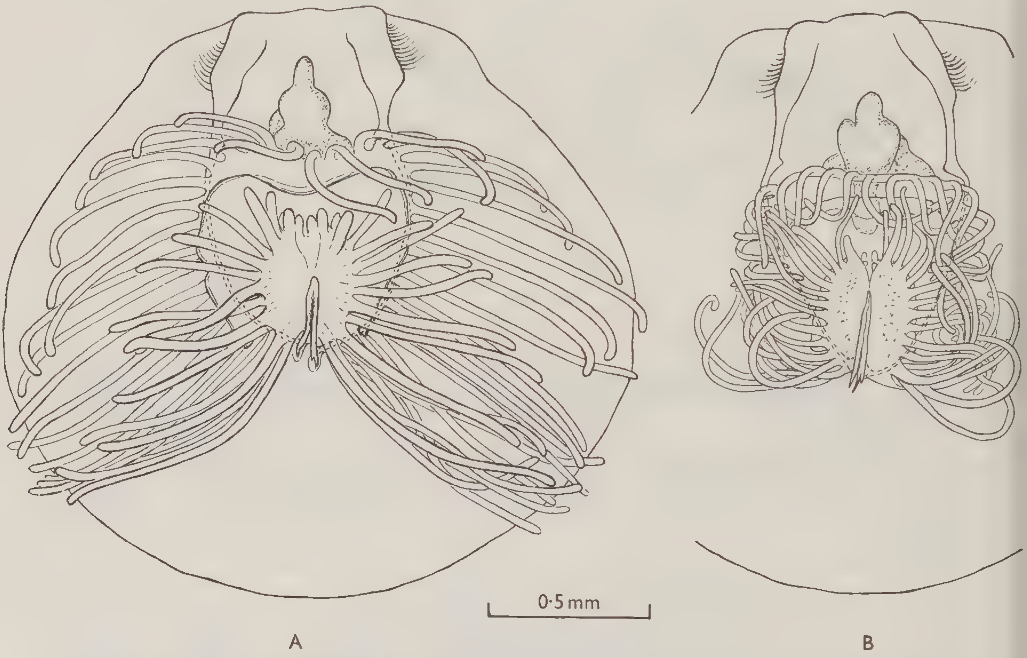


Fig. 5. *M. cranium.* A, Brachial valve with late schizolophe, drawn living. The descending branches—indicated by broken lines—have joined with the septum, which is slightly grooved and has split anteriorly. B, Brachial support more advanced in that the septum is clearly grooved and the descending branches join it at an acute angle. A, B, from separate specimens both of shell length of about 2·2 mm.

At a shell length of 3·5 mm (brachial valve 3·35 mm) the lophophore is zygolophous (Fig. 6). The hood has widened posteriorly and by resorption of its posterior closed end, the transverse band (*t.b.*) has been formed. The anterior spinous processes have increased in number. This stage was drawn from a living individual obtained from La Chapelle Bank region in September 1954. The condition of the brachial support corresponds to Friele's fig. 3.

The transverse sections of this stage, illustrated in Fig. 7, show the relation of the brachial support, and particularly of the hood, to the lateral arms. The somewhat oblique section shown in Fig. 7 A passes through the growing region (*g.r.*) of one side. At this size the great brachial canals (*b.c.g.*) are clearly distinguishable.

At a shell length of 4·2 mm (brachial valve 3·6 mm) the lophophore (Fig. 8 A) is at about the same stage of development as at the smaller shell size (Fig. 6). Growth of the hood, however, has advanced, its lower margins having diverged widely, and is now at about the stage shown in Friele's (1877) fig. 4 (brachial valve 4·5 mm long) and Elliott's (1948) fig. 2 (of shell length 5·5 mm).

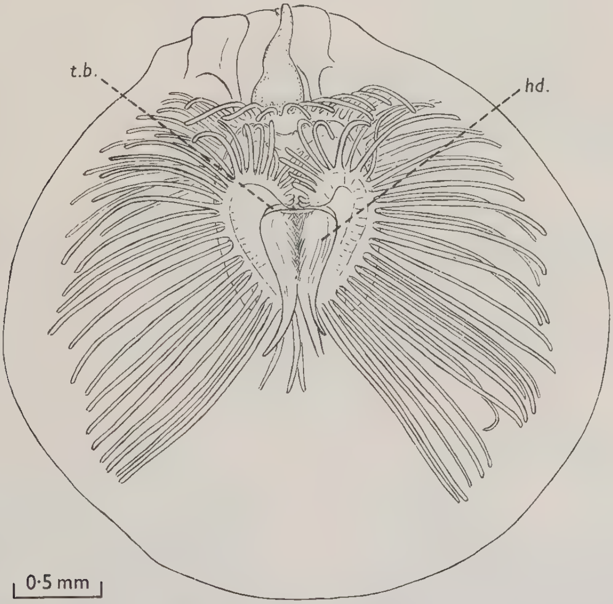


Fig. 6. *M. cranium* of shell length 3·5 mm. Brachial valve with zygolophe, drawn living. The hood (*hd.*) has widened posteriorly and the transverse band (*t.b.*) is distinct. The anterior spinous processes are long. The dorsal filaments of the lateral arms have been omitted so as to avoid confusion.

The last lophophore stage to be described here (Fig. 8 B) is at a shell length of 5·3 mm (brachial valve 4·6 mm). The lophophore is early plectolophous, with one small coil to the spiral arm. In the figure the dorsal part of the spiral arm has been omitted so that the loop should not be entirely hidden. The loop is in the stage shown in Friele's fig. 7, which according to Elliott (1948) corresponds to his figs. 4 and 5. Friele gave the length of his brachial valve as 5·6 mm; Elliott the length of his pedicle valve as 7·0 mm. The freeing of the loop from the septum, and the gradual thinning and disappearance of the

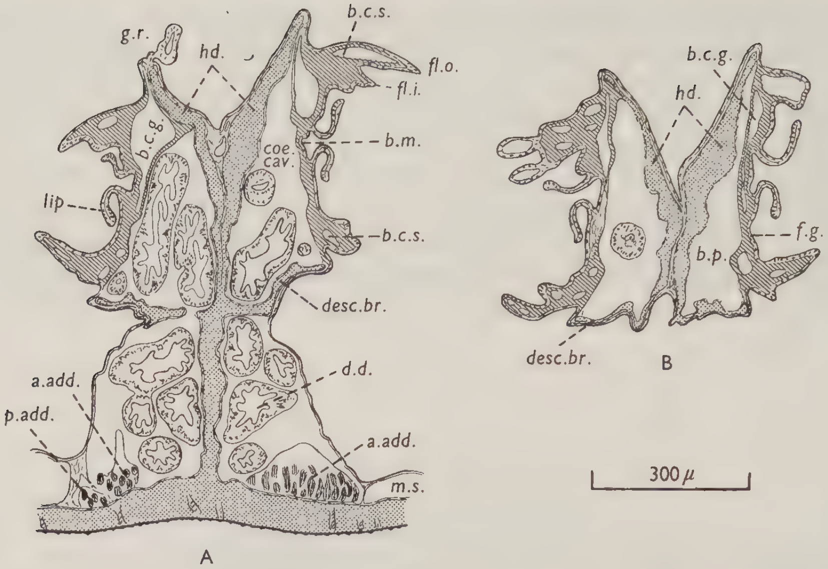


Fig. 7. *M. cranium*. Transverse sections—somewhat oblique—through the brachial valve with zygolophe of about the same stage as shown in Fig. 6, (A) through the hood (*hd.*) and the junction of the right descending branch with the septum, (B) through the lateral arms where free from the body. *a.add.*, anterior adductor muscle; *b.c.g.*, great and *b.c.s.*, small brachial canals; *b.m.*, brachial membrane; *b.p.*, brachial pouch; *coe.cav.*, coelomic cavity; *d.d.*, tubule of digestive diverticulum; *desc.br.*, descending branch of the loop; *f.g.*, food groove; *fl.i.*, inner and *fl.o.*, outer filaments; *g.r.*, growing region of left side; *hd.*, hood; *lip*, lip of the food groove; *m.s.*, mantle sinus; *p.add.*, posterior adductor. Supporting substance shown hatched.

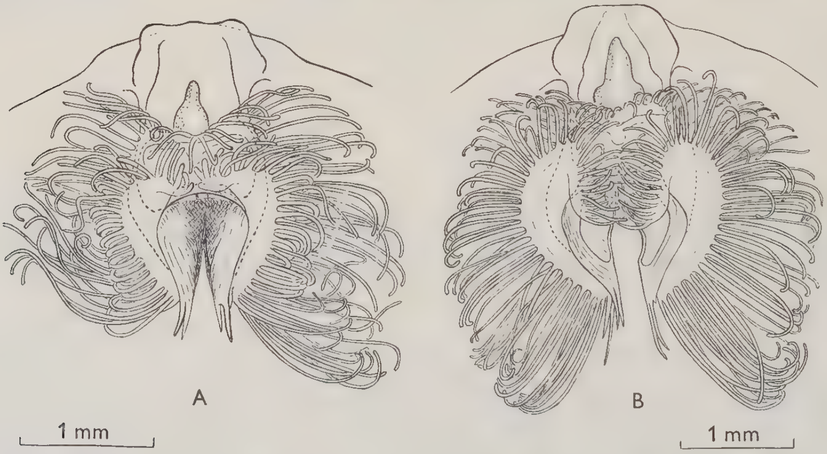


Fig. 8. *M. cranium*. A, Specimen of shell length 4·2 mm: part of the brachial valve with zygolophe, drawn preserved, unnarcotized. The hood is more advanced than that shown in Fig. 6, its lower margins having diverged. B, Specimen of shell length 5·3 mm; early plectolophe with spiral arm of one coil, the dorsal half of which has been omitted for the sake of clearness. The hood shows large lacunae. Preserved after narcotizing and drawn while in cedar wood oil.

latter takes place at this stage; it has been described by Friele (1877, pp. 381–2) and Elliott (1948, p. 301).

Although it has been found that some variation occurs in the shell length at which a certain stage of lophophore and loop is attained, it will have been seen that the loop is in a more advanced stage at a lesser shell size in the La Chapelle Bank specimens than in those of Friele and Elliott. Elliott's *Macandrevia cranium* came from the Hardangerfjord, Norway (1948, p. 316); those of Friele (1877) apparently from the same country.

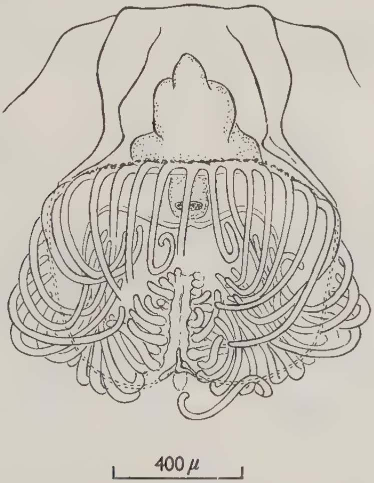


Fig. 9. *M. cranium* of shell length 2·3 mm. Schizolophe with abnormal septum, restricted to a small three-pronged structure at the junction with the descending branches (indicated by broken lines). Stained and drawn while in cedar wood oil.

The development of the brachial support of *M. cranium* is marked by exceptionally early growth of the descending branches of the loop and a late development and early disappearance of the septum.

In *Dallinella* (= *Terebratalia*) *obsoleta*, the only member of the Dallinidae in which the very early stages of the loop have been figured, a septum is present before the development of the crura, and a hood appears on the septum before growth of the descending branches begins (Beecher, 1895).

In *Laqueus californicus*, placed by Konjoukova (1957) in the Dallininae, but by Yabe and Hatai in 1941 (see Muir-Wood, 1955) in the Laqueidae, the septum also appears before the descending branches, as may be seen from her fig. 27 (p. 40).

Small individual with an abnormal septum. One individual of shell length 2·3 mm, with advanced schizolophe, had an abnormally developed septum (Fig. 9). A small calcified region, irregularly three-pronged in shape, occurred at the junction of the descending branches with it. The remainder of the septum appeared to be represented by connective tissue only. The median dorsal mesentery was clearly visible running up its centre.

THE ADULT LOPHOPHORE

The adult lophophore is plectolophous, supported by a long calcareous loop: spicules are entirely lacking. As the shell is long the lateral and spiral arms are long. The loop extends to the extreme end of each lateral arm, and a spine projects anteriorly. The position of the twin growing regions at the apex of the spiral arm is shown in transverse section in Fig. 10A. From the sections illustrated in Fig. 10 the very largely tubular structure of the lophophore of

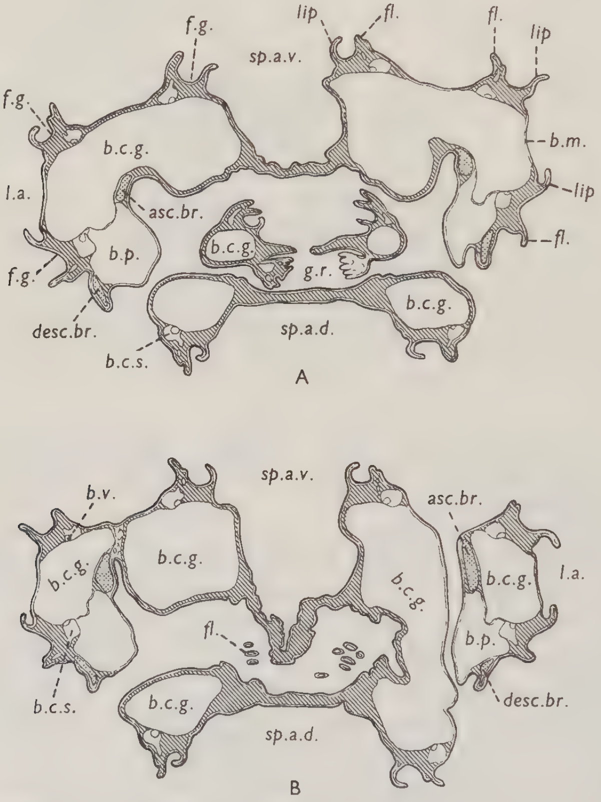


Fig. 10. *M. cranium* of shell length 17 mm. Transverse sections through adult plectolophe. A, Section through the twin growing regions of the lophophore (*g.r.*) at the apex of the spiral arm. The great brachial canals of the lateral and spiral arms are in communication. B, a section anterior to A, with the spiral and lateral arms becoming separate. *asc.br.*, ascending branch of the loop; *b.c.g.*, great and *b.c.s.*, small brachial canals; *b.m.*, brachial membrane; *b.p.*, brachial pouch; *b.v.*, 'blood' vessel; *desc.br.*, descending branch of the loop; *f.g.*, food groove; *fl.*, filament; *l.a.*, lateral arm; *lip*, lip of the food groove; *sp.a.d.*, dorsal and *sp.a.v.*, ventral side of first coil of spiral arm. Supporting substance shown hatched.

Macandrevia cranium is apparent. There is so little tissue that on fixation a certain amount of distortion occurs, for instance the brachial membrane connecting the opposite rows of filaments of the ventral part of the first coil of the spiral arm is probably too deeply indented.

The usual canals are present in the lophophore. Small brachial canals (*b.c.s.* Figs. 10, 11), arising from the perioesophageal lacunae, run at the bases of the filaments to which they give off branches. Within the small brachial and the filamentar canals are 'blood vessels', with fine muscle fibres in their walls. The great brachial canals (*b.c.g.*) each ends in a blind sac on each side of the oesophagus (see also Hancock, 1858, p. 807). In the lateral arms they do not extend as far anteriorly as do the small brachial canals, which are continuous around the blind end of the arms. Posteriorly the great brachial canals of the lateral and spiral arms are continuous (Fig. 10A), as noted by Hancock; anteriorly they become separate (Fig. 10B). In each lateral arm a brachial pouch, a continuation of the coelomic cavity, extends anteriorly to its extremity, reaching beyond the great and small brachial canals. In Figs. 10 and 11 it is impossible at the magnification shown to indicate the various tissues.

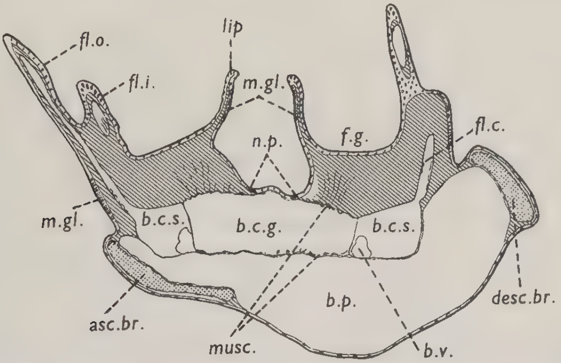


Fig. 11. *M. cranium.* Transverse sections of lateral arm of an adult. *fl.c.*, filamentar canal; *fl.i*, inner and *fl.o.*, outer filaments; *m.gl.*, mucous gland cells; *musc.*, muscle fibres; *n.p.*, principal nerve. Other lettering as in Fig. 10.

Muscle fibres are present in the walls of the canals, chiefly beneath the supporting substance under the food grooves and the filaments. Here they are mainly transverse in direction and on contraction compress the supporting substance, so that in sections it appears to have fibres running normal to the food groove. Contraction of these muscle fibres also causes the brachial membrane to be thrown into folds. In the lip of the food groove muscle fibres are present below the epithelium.

Nerves are difficult to distinguish in sections of the lophophore, indeed only the principal nerve (*n.p.*) could be certainly identified: its position is shown in Fig. 11.

The distribution of mucous gland cells on the lophophore is similar to that in other plectolophes examined. They occur: (1) in an abfrontal tract below the bases of the filaments; (2) on the brachial membrane of the arms; and (3) on the walls, including the lip of the food groove.

The filaments are in the usual double alternating series, the outer with grooved and the inner with ridged frontal surfaces. Behind the mouth twelve to fourteen pairs of filaments are in single series and have ridged frontal surfaces: these filaments are short. Longitudinal muscle fibres are present in the walls of the filamentar canals, chiefly in a frontal group, in which the fibres are striated.

The body of the lophophore is entirely ciliated, except possibly for mucous cells. The outer surfaces of the filaments are entirely ciliated, the cilia being in four tracts: frontal, abfrontal and paired lateral.

The ciliary feeding mechanism of *M. cranium* has been briefly described (Atkins, 1956) and it is intended to publish a full account separately.

In the various growth stages resulting in the plectolophe of the adult, new filaments are formed in two immediately adjacent regions, one on each side of the mid-line. The two growing regions remain close together in all developmental stages and in the final plectolophe are situated at the apex of the spiral arm. There is no interpolation of filaments, and yet the lateral arms increase in length commensurate with growth in length of the shell. It appears that there is a shifting around of the filaments, together with the underlying brachial canals, so that those on the ventral side of the lateral arms move around on to the dorsal side, while filaments which were originally situated on the spiral arm will move around on to the ventral side of the lateral arm. The two opposite rows of filaments of the spiral arm are distinct in that one set belongs to the left side of the body and one to the right: there are two distinct great brachial canals. In the lateral arms there is a single great brachial canal for the two rows of filaments on opposite sides of the arms: the two tubes having as it were coalesced (Hancock, 1858).

To account for the forward growth of the lateral arms in the lophophore of terebratulaceans and terebratellaceans, Williams (1956, pp. 261–3) suggested that growth in the median zone is temporarily suppressed, while two secondary growth regions arise in the antero-lateral positions of the lophophore. This suggestion seems to be unfounded. Secondary growth zones would presumably be marked by the presence of short filaments and filament buds, but such have been found only in the twin growing regions in the median indentation of the schizolophe in all brachiopods studied, including *Terebratella inconspicua* which Williams figured (1956, fig. 5 (1)). And far from growth being suppressed in the median zone, it becomes accelerated, as the presence of crowded filament buds shows (see Fig. 2). The growth of new filaments appears to be so rapid that their migration anteriorly and laterally to form the lateral arms does not keep pace with it, and the growing regions turn either anteriorly, and finally ventro-dorsally, as in plectolophes, or laterally, as in spirolophes, encroachment on the mouth thus being avoided.

I am grateful to Dr L. H. N. Cooper for having the two dredge hauls—which contained the tiny brachiopods—taken while on hydrographical cruises. My thanks are due to Dr J. S. Alexandrowicz for translating the Russian of Konjoukova for me, and to Mr G. F. Elliott for reading the manuscript.

A grant from the Browne Research Fund of the Royal Society for the year 1956–57 and a London University table made the work possible.

SUMMARY

The early growth stages of lophophore and loop of *Macandrevia cranium* at a shell length of 1·0–5·3 mm are described. Figures are given to show that in this dallinid the descending branches of the loop grow from the crura only and fuse with the septum. The figures also show that only the two primary growth regions persist, and that no secondary growth zones arise.

The growth of the brachial support is marked by exceptionally early development of the descending branches of the loop and a late development and early disappearance of the septum.

The structure of the adult plectolophous lophophore is described.

REFERENCES

Atkins, D., 1956. Ciliary feeding mechanisms of brachiopods. *Nature, Lond.*, Vol. 177, pp. 706–7.

—— 1959. The growth stages of the lophophore of the brachiopods *Platidia davidsoni* (Eudes Deslongchamps) and *P. anomioides* (Philippi), with notes on the feeding mechanism. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 103–32.

Beecher, C. E., 1895. The development of *Terebratalia obsoleta* Dall. *Trans. Conn. Acad. Arts Sci.*, Vol. 9, pp. 392–9.

Elliott, G. F. 1948. The evolutionary significance of brachial development in terebratelloid brachiopods. *Ann. Mag. nat. Hist.*, Ser. 12, Vol. 1, pp. 297–317.

—— 1953. Brachial development and evolution in terebratelloid brachiopods. *Biol. Rev.*, Vol. 28, pp. 261–79.

Friele, H., 1877. The development of the skeleton in the Genus *Waldheimia*. *Arch. Math. Naturv.*, Vol. 2, pp. 380–6.

Hancock, A., 1858. On the organization of the Brachiopoda. *Phil. Trans.*, Vol. 148, pp. 791–869.

Konjoukova, E. D., 1948. The post-embryonic development of the shell and of the brachial apparatus of certain Pacific brachiopods. *Bull. Acad. Sci. U.S.S.R. (Biol.)*, No. 2, p. 213. (Not seen).

—— 1957. Brachiopods of far eastern seas. *Invest. Far Eastern Seas U.S.S.R.*, Vol. 4, pp. 5–84.

Muir-Wood, H. M., 1955. *A History of the Classification of the Phylum Brachiopoda.* 124 pp. London: Brit. Mus. (Nat. Hist.).

Percival, E., 1944. A contribution to the life-history of the brachiopod, *Terebratella inconspicua* Sowerby. *Trans. roy. Soc. N.Z.*, Vol. 74., pp. 1–23.

Thomson, J. A., 1927. Brachiopod morphology and genera (Recent and Tertiary). *N.Z. Bd Sci. Art*, Manual 7.

Williams, A., 1956. The calcareous shell of the Brachiopoda and its importance to their classification. *Biol. Rev.*, Vol. 31, pp. 243–87.

J. mar. biol. Ass. U.K. (1959) 38, 351–359
Printed in Great Britain

MOULTING HORMONES IN *PALAEMON* (=*LEANDER*) (CRUSTACEA DECAPODA)

II. DIFFERENCES BETWEEN POPULATIONS

By D. B. Carlisle
The Plymouth Laboratory

(Text-figs. 1–3)

Eye-stalk removal in certain species of Brachyura and Astacura has almost invariably led to the initiation of proecdysis. In other decapod crustaceans, however, there have been significant variations in the results of this operation. Thus Travis (1951) found that eye-stalk ablation had no effect on the duration of the moult cycle in *Panulirus argus*. And in the Natantia, even in a single species, quite different results have been reported. Drach (1944) found that eye-stalk removal in *Palaemon* (= *Leander*) *serratus* (Pennant) led, as in crabs, to the initiation of proecdysis and to accelerated moulting. In ignorance of this work I repeated some of the same experiments and found that eye-stalk removal in *P. serratus* led to slower moulting (Carlisle, 1953*a*)—a result quite opposite to that of Drach. Scheer & Scheer (1954) confirm my results in the same species.

Three main types of hypothesis may be advanced to explain these contradictions: (1) that the conditions were different in the laboratories of the three groups of workers, that is to say in the laboratories of Roscoff, Plymouth and Naples; (2) that the experimental techniques adopted were different—for instance, animals might be fed or starved; (3) that the population of prawns in the three laboratories might be different and the differing results be inherent in the material.

In order to decide between these hypotheses some hundreds of *Palaemon serratus* collected at Roscoff—the laboratory where Drach performed his experiments—were brought to Plymouth and duplicate experiments were performed on these and on indigenous Plymouth specimens.

At the outset it is worthy of remark that after a little practice it was possible to distinguish between the prawns from Plymouth and from Roscoff on the basis of the colour patterns (Carlisle, 1955). Certainly about 30% of each population could be confused, but the remaining 70% were clearly different. Moreover, in colour photographs of Naples prawns, taken by Sir Francis Knowles, it was easily seen that these prawns were different again. The differences were slight and not such as would satisfy a systematist for specific, or even subspecific, characterization, but they were nevertheless real, despite the overlap between the populations.

EXPERIMENTAL DATA AND CONCLUSIONS

The prawns were kept in running sea water in the aquarium tanks at Plymouth, either as a group of prawns in a large tank or singly in cages. The cages were made of $\frac{1}{4}$ in. mesh stainless steel, and each was 1 m square by 10 cm deep and divided into 100 cubical compartments of 10 cm side. A single prawn was placed in each compartment and the whole cage lowered into an aquarium tank. Prawns were fed daily on the flesh of *Mytilus*. For experimental purposes prawns were selected between 55 and 70 mm length measured from the tip of the rostrum to the tip of the telson. Eye-stalk ablation was performed by electrocautery; a single eye-stalk was removed on one day and the other on the succeeding day. Under these conditions the survival rate throughout the experiment was better than in earlier experiments (see Carlisle, 1953*a*).

Four groups of prawns were used: group P1—Plymouth prawns with eye-stalks removed; group P2—intact Plymouth prawns; group R1—Roscoff prawns with eye-stalks removed; group R2—intact Roscoff prawns.

In Fig. 1 is illustrated the variation of intermoult period in these four groups with alteration in temperature (small symbols). The points for the Plymouth prawns represent my own earlier experiments and those for the Roscoff prawns are plotted from the data of Drach (1944). The lines were drawn through the points by eye.

In the first experiment with these four groups of prawns a number of prawns were kept through two successive moults and the interval between these moults recorded. The temperature throughout this experiment was $13{\cdot}7 \pm 1{\cdot}1^\circ$ C. The results are summarized in Table 1 and diagrammatically in Fig. 2. The mean intermoult periods for the four groups are:

$$\bar{t}_{\mathrm{P1}} = 51{\cdot}140 \pm 0.933, \quad \bar{t}_{\mathrm{P2}} = 35{\cdot}513 \pm 0{\cdot}715,$$
$$\bar{t}_{\mathrm{R1}} = 17{\cdot}356 \pm 0{\cdot}494, \quad \bar{t}_{\mathrm{R2}} = 22{\cdot}206 \pm 0{\cdot}492.$$

where t is time in days. From this it may be calculated that the probability, P, that these four groups are drawn from the same population is less than 0·001. For all the individual comparisons, except $\bar{t}_{\mathrm{R1}} \sim \bar{t}_{\mathrm{R2}}$, $P < 0{\cdot}001$; for $\bar{t}_{\mathrm{R1}} \sim \bar{t}_{\mathrm{R2}}$, $P = 0{\cdot}001$. It is thus quite evident that all four groups are very significantly different. These four means are entered into Fig. 1 as the four large symbols in line with the temperature 13·7° C. It will be seen that they fit well with the curves drawn from the earlier data.

In the second experiment a larger number of prawns was used for a shorter period and the moulting rate followed over 15 days. The temperature during this experiment was 12·1 ± 0·4° C. The numbers of deaths and of moults in each group on each day of the experiment are listed in Table 2 and the moult rate illustrated graphically in Fig. 3. From these data mean intermoult periods can be calculated for the four groups:

$$\bar{t}_{\mathrm{P1}} = 103{\cdot}077,\ \bar{t}_{\mathrm{P2}} = 42{\cdot}118,$$
$$\bar{t}_{\mathrm{R1}} = 17{\cdot}092,\ \bar{t}_{\mathrm{R2}} = 23{\cdot}567$$

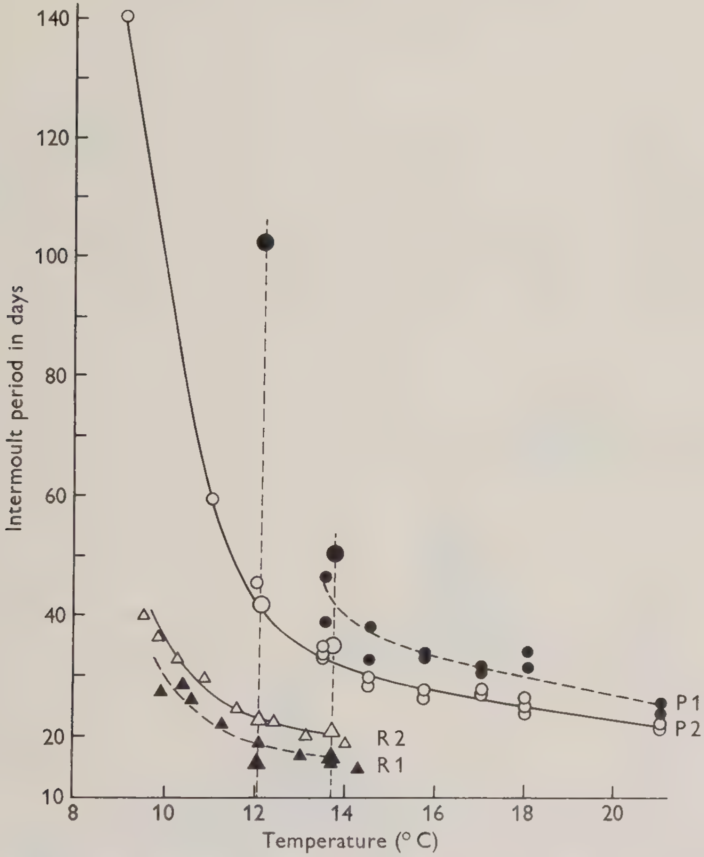


Fig. 1. Graph of the intermoult period of *Palaemon serratus* plotted against temperature. ●, P1: prawns from Plymouth whose eye-stalks had been removed; ○, P2: intact prawns from Plymouth; ▲, R1: prawns from Roscoff whose eye-stalks had been removed; △, R2: intact prawns from Roscoff. The lines are drawn through the small symbols by eye. Lines P1 and P2 represent my data; lines R1 and R2 are replotted from the data of Drach (1944). For the large symbols see the text.

These means are entered into Fig. 1 as the four large points in line with temperature 12·1° C. Once more the fit of the curves from the earlier data, extrapolated where necessary, is good. To calculate the significance of the differences in the moult rates in this experiment it is necessary to compute

TABLE 1

Days between successive moults	Number of prawns in Group P1	Group P2	Group R1	Group R2
10	.	.	1	.
11	.	.	2	.
12	.	.	1	.
13	.	.	4	.
14	.	.	5	1
15	.	.	3	1
16	.	.	7	2
17	.	.	12	1
18	.	.	5	3
19	.	.	8	8
20	.	.	2	6
21	.	.	3	11
22	.	.	1	10
23	.	1	2	5
24	.	.	.	6
25	.	1	.	3
26	.	4	1	4
27	.	.	.	1
28	.	3	.	2
29	.	1	2	.
30	.	2	.	.
31	.	3	.	1
32	.	4		.
33	.	10	.	.
34	.	9	.	2
35	.	13	.	1
36	1	5	.	.
37	.	7	.	.
38	1	3	.	.
39	.	5	.	.
40	.	.	.	.
41	1	1	.	.
42	2	.	.	.
43	.	4	.	.
44	1	1	.	.
45	2	1	.	.
46	3	.	.	.
47	3	.	.	.
48	4	.	.	.
49	6	.	.	.
50	7	.	.	.
51	6	1	.	.
52	5	.	.	
53	2	.	.	.
54	1	.	.	.
55	1	.	.	.
56	3	2	.	.
57	.	.	.	.
58	.	.	.	.
59	1	.	.	.
60	.	.	.	.
61	2	.	.	
62	1	1	.	.
63	.	.	.	.
64	1	.	.	.
65	.	.	.	.
66	.	.	.	.
67	.	.	.	.
68	1	.	.	.
69	1	.	.	.
70	.	.	.	.
71	.	.	.	.
72	.	.	.	.
73		.	.	.
74	1	.	.	.

parallel probit lines and obtain probabilities by the modified mean probit difference method outlined by Carlisle & Dohrn (1953). The various possible comparisons and the corresponding probabilities are:

$$\begin{aligned}
\Delta(a_{\mathrm{P1}} \sim a_{\mathrm{P2}}) &= 0{\cdot}47 \pm 0{\cdot}14;\ P < 0{\cdot}001,\\
\Delta(a_{\mathrm{P1}} \sim a_{\mathrm{R1}}) &= 0{\cdot}97 \pm 0{\cdot}15;\ P < 0{\cdot}001,\\
\Delta(a_{\mathrm{P1}} \sim a_{\mathrm{R2}}) &= 0{\cdot}79 \pm 0{\cdot}14;\ P < 0{\cdot}001,\\
\Delta(a_{\mathrm{P2}} \sim a_{\mathrm{R1}}) &= 0{\cdot}50 \pm 0{\cdot}12;\ P < 0{\cdot}001,\\
\Delta(a_{\mathrm{P2}} \sim a_{\mathrm{R2}}) &= 0{\cdot}32 \pm 0{\cdot}11;\ P < 0{\cdot}01,\\
\Delta(a_{\mathrm{R1}} \sim a_{\mathrm{R2}}) &= 0{\cdot}18 \pm 0{\cdot}11;\ P = 0{\cdot}1,
\end{aligned}$$

where a is the mean probit and Δ the mean probit difference. All these differences except the last were highly significant.

We may conclude from these two experiments that the differences found between the four groups of prawns by independent workers in different laboratories are genuine differences inherent in the prawns, and not merely an expression of differing conditions or differing experimental techniques.

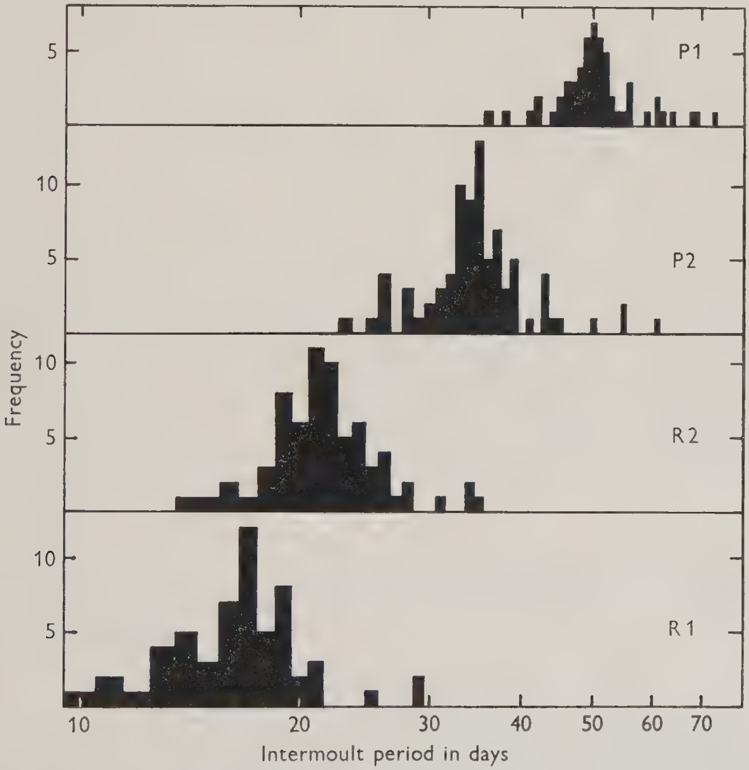


Fig. 2. Histograms drawn from the data of Expt. 1. The four groups of prawns are numbered as in Fig. 1. The time scale is logarithmic. Temperature 13·7° C.

TABLE 2. DEATHS AND MOULTS IN EXPERIMENT II

The number of animals which died without first moulting and of those which moulted on each day in each group.

Group...	P1		P2		R1		R2	
No. of animals...	205		301		198		297	
Day	Dead	Moults	Dead	Moults	Dead	Moults	Dead	Moults
1	5	2	2	6	3	7	3	4
2	3	1	5	6	4	8	5	10
3	.	1	.	8	3	9	.	17
4	3	3	.	3	2	9	.	5
5	.	1	.	4	.	7	2	7
6	.	2	1	5	.	7	.	8
7	2	.	.	4	2	8	.	6
8	.	3	.	9	.	5	.	10
9	.	.	.	8	3	8	1	11
10	2	5	3	5	.	6	.	13
11	1	.	.	5	1	6	.	8
12	.	1	.	4	.	7	1	9
13	.	3	1	7	.	6	.	12
14	.	2	.	4	2	10	1	7
15	1	2	.	7	.	6	.	7
Totals	17	26	12	85	20	109	13	134

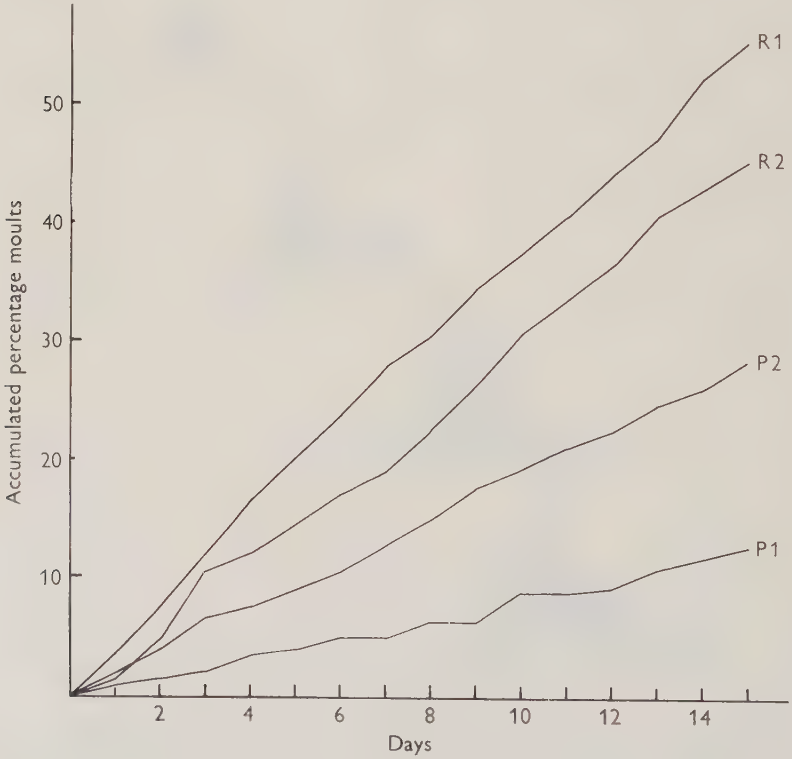


Fig. 3. Graph of the cumulative percentage of moults in Expt. 2. The four groups of prawns are numbered as in Fig. 1. Temperature 12·1° C.

There is a real difference in moult rate between intact *Palaemon serratus* from Plymouth and from Roscoff and the operation of eye-stalk ablation performed on these two populations has quite opposite effects leading to a longer intermoult period in Plymouth animals and to a shorter intermoult period in Roscoff prawns.

DISCUSSION

There can be little doubt that the results of the experiments of Drach (1944) indicate the existence of a moult-inhibiting hormone in the eye-stalks of *Palaemon serratus.* On the other hand, the results of Carlisle & Dohrn (1953) and Scheer & Scheer (1954) indicate the presence of a moult-accelerating hormone in the eye-stalks of *Palaemon*, a hormone which accelerates the processes of proecdysis. It must not be thought that these two hormones are mutually antagonistic, for the moult inhibiting hormone inhibits the onset of proecdysis, while the moult accelerating hormone accelerates the progress of proecdysis once this has begun. Both hormones, however, will affect the duration of the intermoult period; the moult inhibiting hormone by lengthening diecdysis will increase the intermoult period, while the moult-accelerating hormone by shortening proecdysis will shorten the intermoult period. Thus, in a certain measure, the length of the intermoult period will depend on the relative rates of secretion of these two hormones, and on the rates of secretion of the moult-inhibiting and moult-accelerating hormones which are known to be produced in other parts of the body (Carlisle, 1953*b*, 1954; Scudamore, 1947; Stephens, 1951). The effects of eye-stalk ablation on the duration of the intermoult period will also depend on the relative rates of secretion of these various hormones. Here then we have a possible mechanism for the different moult rates in two populations of prawns and for the opposite effects that eye-stalk ablation has on these two populations. Presumably the differences between the populations are genotypic, for besides the differences in moult rate there are also differences in colour pattern and even in the precise topography of the endocrine organs of the eye-stalk—those organs which are responsible for the secretion of the moulting hormones. This is true also for Naples prawns which are different in both these respects from Plymouth and from Roscoff prawns.

I wish to thank Prof. P. Drach for his helpful criticisms and comments and the staff and fishermen of the Station Biologique de Roscoff for their friendly collaboration.

SUMMARY

The populations of *Palaemon* (= *Leander*) *serratus* from Roscoff, Plymouth and Naples are noticeably different in the range of colour patterns, though there is some overlap between the populations from Plymouth and Roscoff at least. The mean intermoult period at any one temperature is different for these

two populations, even if kept in adjacent tanks in the Plymouth aquarium; the intermoult period is shorter for prawns from Roscoff. Eyestalk ablation leads to significant shortening of the intermoult period in prawns from Roscoff but to significant lengthening of this period in prawns from Plymouth, even when duplicate experiments are carried out in the one laboratory on collections of prawns from the two localities. An endocrinological explanation of these experiments is proposed.

REFERENCES

CARLISLE, D. B., 1953*a*. Moulting hormones in *Leander* (Crustacea Decapoda). *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 289–95.

—— 1953*b*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). IV. On the site of origin of the moult-accelerating principle—experimental evidence. *Publ. Staz. zool. Napoli*, Vol. 24, pp. 285–92.

—— 1954. On the hormonal inhibition of moulting in decapod Crustacea. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 61–3.

—— 1955. Local variations in the colour pattern of the prawn *Leander serratus* (Pennant). *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 559–63.

CARLISLE, D. B. & DOHRN, P. F. R., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). II. Experimental evidence for a growth- and moult-accelerating factor obtainable from eyestalks. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 69–83.

DRACH, P., 1944. Étude préliminaire sur le cycle d'intermue et son conditionnement hormonal chez *Leander serratus* (Pennant). *Bull. biol.*, Vol. 78, 40–62.

SCHEER, B. T. & SCHEER, M. A. R., 1954. The hormonal control of metabolism in crustaceans. VII. Moulting and colour change in the shrimp *Leander serratus*. *Pubbl. Staz. zool. Napoli*, Vol. 25, pp. 397–418.

SCUDAMORE, H. H., 1947. The influence of the sinus glands upon molting and associated changes in the crayfish. *Physiol. Zoöl.*, Vol. 20, pp. 187–28.

STEPHENS, G. C., 1951. A molt-inhibiting factor in the central nervous system of the crayfish, *Cambarus* sp. *Anat. Rec.*, Vol. 111, pp. 572–73.

TRAVIS, D. F., 1951. The control of the sinus glands over certain aspects of calcium metabolism in *Panulirus argus* Latreille. *Anat. Rec.*, Vol. 111, p. 503.

APPENDIX

Some of the data from which curves P1 and P2 in Fig. 1 are drawn have been published (Carlisle, 1953*a*). Most of them, however, have not. As explained in the paper mentioned above it is possible to measure the intermoult period either directly or by calculation from the moult rate measured in a group of prawns over a period of days. By the latter method it is not possible to assign a standard error to the mean intermoult period, although it is possible to assign one to the difference between moult rates. Points on the graph (Fig. 1) have been obtained by both these methods. In Table 3 are given the mean intermoult periods corresponding to each of the points on curves P1 and P2, together with a standard error where this is appropriate.

TABLE 3. INTERMOULT PERIODS

Table of the data which generated the curves P1 and P2 in Fig. 1. Where a standard error of the mean is given the mean intermoult period was derived from a number of direct observations of individual intermoult periods. Where no standard error is given the intermoult period is calculated from the moult rate measured in a number of animals over the stated number of days.

	P1		P2	
Temperature (° C)	Intermoult period (days)	Number	Intermoult period (days)	Number
9·0	.	.	141·12 ± 11·27	93
10·0	.	.	94·63 ± 11·01	112
11·0	.	.	60·10 ± 7·33	108
12·0	.	.	45·95 ± 4·95	101
13·5	39·40 ± 2·71 35·46	5 (100 × 26 days)	33·78 ± 1·58 34·62 ± 3·19 47·30	9 127 (100 × 26 days)
14·5	33·10 38·74 ± 3·01	(150 × 15 days) 16	29·13 ± 2·01 30·10	18 (150 × 15 days)
15·8	33·87 34·30 ± 2·76	(100 × 15 days) 9	26·28 ± 1·99 27·00	12 (100 × 15 days)
17·1	31·12 ± 2·81 32·13	15 (100 × 15 days)	27·22 27·84 ± 2·02	(100 × 15 days) 20
18·0	31·94 ± 2·11 34·50	21 (100 × 15 days)	24·23 ± 0·76 25·66 ± 1·94 26·47	132 24 (100 × 15 days)
21	24·11 ± 0·83 25·97	33 (100 × 15 days)	21·81 22·79 ± 0·68	(100 × 15 days) 35

J. mar. biol. Ass. U.K. (1959) 38, 361–367
Printed in Great Britain

THE ECOLOGY OF *ECHINUS ESCULENTUS* L. QUANTITATIVE DISTRIBUTION AND RATE OF FEEDING

By G. R. Forster
The Plymouth Laboratory

(With Plates I and II and Text-figs. 1 and 2)

In order to provide more factual information on the effect of *Echinus* predation (Forster, 1958), further diving observations have been made during 1958. A rocky area just west of the Tinker Shoal was explored by chance and a population of *Echinus* discovered. As this area is much more readily accessible than Stoke Point rock, where most of the diving has been carried out, it was possible to establish a marker buoy and make a number of dives on exactly the same position. In the course of these dives I have attempted to measure the numerical density of the urchins and the rate of their browsing on the rock surface. Later *Echinus* 'counts' were made at many other positions near Plymouth (Text-fig. 1).

The abundance of *Echinus* has been measured by counting the number of urchins observed in a known area of sea-floor. A terylene line roughly 50 m long is laid out on a rocky part of the sea bed as is shown diagrammatically (Text-fig. 2). One end of the line is attached to the bottom of a shot rope or to the boat's anchor. The line is then drawn out to its full length with the aid of a dinghy. The diver swims along the line with a $2\frac{1}{2}$ m rod ($\frac{5}{16}$ in. galvanized wire) held at right angle to the line, counting the numbers of urchins first on one side of the line and returning along the other side; in this way a strip of rock surface 5 m wide is covered. If the line is found to be suspended between pinnacles of rock the diver can lift the 7 lb. lead at the distant end and drag it slightly until the line just touches the rocks' surface. Should part of the line be lying on sand, the actual area where urchins are being counted can be marked off by tying knots in the line.

The rate of browsing was measured by turning over a few large boulders in a position where *Echinus* were present, and placing one urchin on the newly exposed surface of each boulder. On the next dive 24 h later, if the *Echinus* was still occupying a position on the boulder, it was removed and the outline of the area which had been browsed was traced on a sheet of Ethulon. It was hoped that the undersides of these boulders, usually covered with many small barnacles (*Verruca stroemia*) would prove attractive to the *Echinus*, since *Verruca* are readily browsed on by urchins in an aquarium tank. In addition a pair of wire frames covered with large mesh nylon netting have been used to confine a number of urchins in a small area. These frames were 1 m^2 in area and about 18 in. high. A heavy chain was laced to the edge of the netting to keep it in contact with the rock all the way round, also serving to prevent the frame being moved about by wave action. After a period of several weeks the browsed area under the frame can be compared with a control area under the second frame from which *Echinus* have been excluded, or simply with the immediate surrounding rock surface if this has been kept clear of urchins during the course of the experiment.

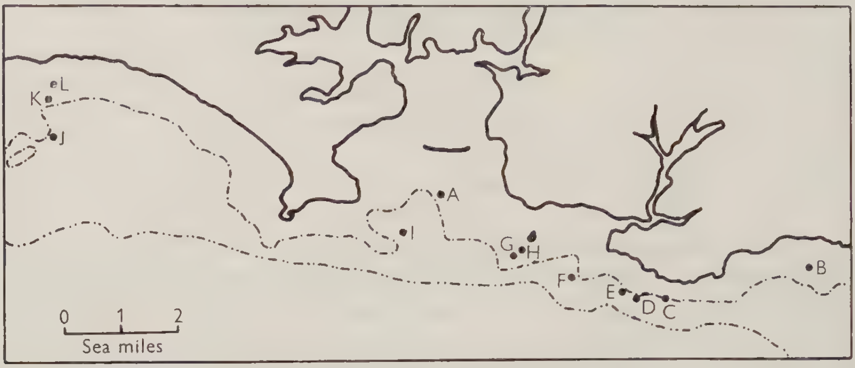


Fig. 1. Outline chart of Plymouth Sound and neighbourhood showing positions A–L, described in Table 1. The 10 and 20 fm lines are represented by lines with single and double dots respectively.

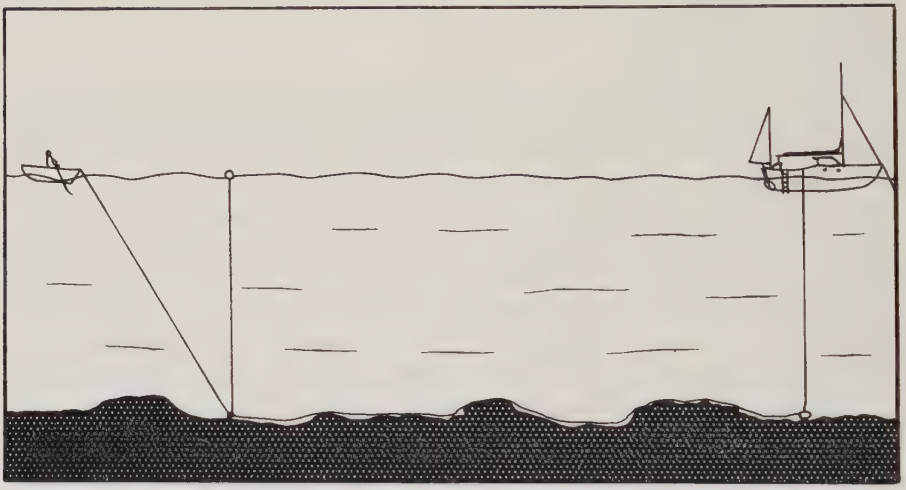


Fig. 2. Diagram showing the method of laying out the 50 m line over the rocky sea floor.

Some tests on the browsing of *Echinus* in aquarium tanks have also been made. The area of algal film swept clear by an urchin over a known period can be marked on the glass surface or traced directly on to Ethulon so that the area can be measured. The urchins used in these tests had all been allowed to acclimatize themselves to aquarium conditions for several weeks.

RESULTS

Table I shows the results of *Echinus* counts made chiefly during September and October 1958.

The average distribution of *Echinus* in the areas examined is therefore one per $4\frac{2}{3}$ m^2, or 868 per acre. Thus in the 4 mile strip of coast between the Mewstone and Revelstoke Point, it may be assumed from the chart that there is generally about $\frac{1}{2}$ mile separating the 7 fm from 17 fm contour; in this narrow strip alone there should be a population of nearly 1,400,000 urchins.

All the underwater observations on browsing were made in the immediate vicinity of a marker buoy situated 4 cables west of the Tinker Shoal. The marker buoy cable was attached to a projecting rock on the bottom. The following transits make it a simple matter to locate the position if the buoy should be submerged or lost. The higher anchorage beacon on Ramscliff Point is in line with the beacon on the east end of the breakwater, and the north-west corner of the Mewstone appears to be almost touching the southernmost tip of Stoke (Hilsea) Point.

The results of tests on the rate of browsing are shown in Table 2. The algal films in the last two tests were composed of *Hildenbrandia prototypus* Nardo (red) and a mixture of various green and blue-green algae. The average browsing rate for the 8 tests is 2·9 cm^2/h.

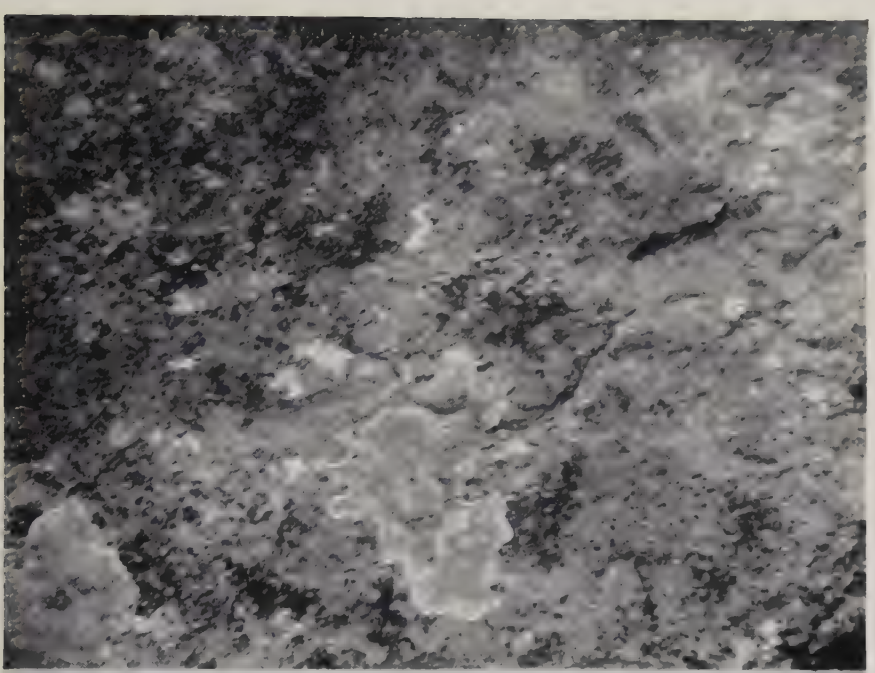
GENERAL NOTES ON BROWSING AND HABITS

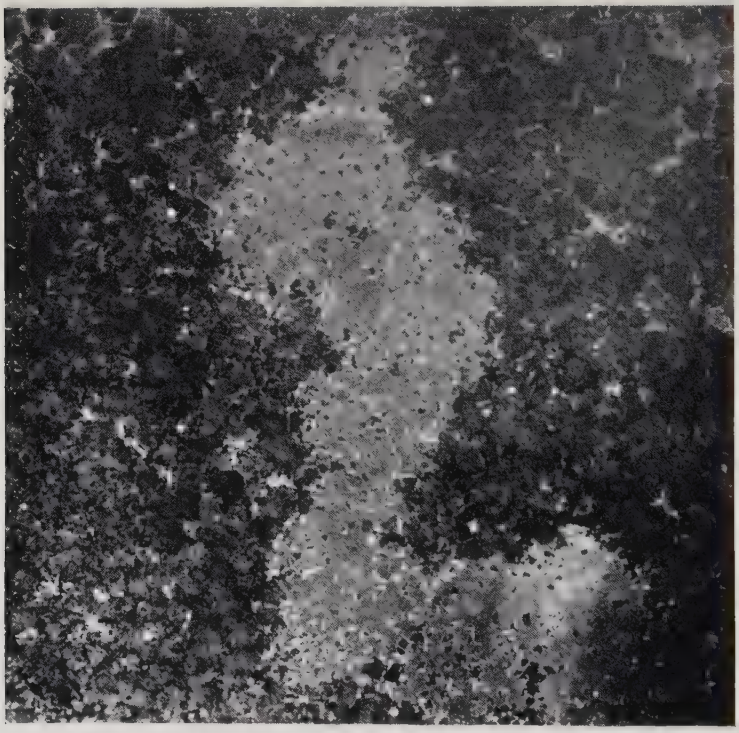
Underwater observations

A browsed area of about $\frac{1}{4}$ to $\frac{1}{2}$ m^2 is usually recognizable around most of the *Echinus* examined underwater. This is not completely bare rock but is devoid of any normal brown 'fur' composed of very small filamentous algal growths encrusted by diatoms, etc., the remains of hydroid colonies similarly overgrown besides muddy tubes of amphipods and various polychaetes; it can best be described as presenting a clean 'scrubbed' appearance. In Plate I two underwater photographs show the contrast between browsed and normal rock surfaces. The browsed area formed a small part of the rock covered by a netting frame which confined six *Echinus* to 1 m^2 and was photographed after a month. The second photograph shows the normal appearance of the rock and was taken about 1–2 m away from the netting frame. *Lithothamnion* is usually very common in the browsed areas. Whether this browsed area is in any way similar to the territory of a limpet is not yet known. It will also be of interest to ascertain if there is any tendency for the *Echinus* to home

TABLE 1

	Position	Depth (fm)	Length measuring line (m)	Area (m²)	No. *Echinus*	Notes
A	4 cables west from Tinker Shoa	8½–9	54	135	14	Line arranged east from marker buoy
		8½–9	54	135	11	
		8½–9	54	135	22	Line arranged north from marker buoy
		8½–9	54	135	14	
		8½–9	54	135	20	Line arranged south from marker buoy
		8½–9	54	135	4	
		8½–9	54	135	8	
		8½–9	54	135	18	Line arranged west from marker buoy
		8½–9	54	135	6	
		8½–9	50	125	17	Line running along a gully wall
B	Bigbury Bay ½ mile south of St Anchorite's rock	9	36	90	2	Line often close to or cutting across gravel patches
		9	36	90	5	
C	About 30 yards south-west from Stoke Pt (=Hilsea Pt) rock	14–15	54	135	36	Normal rock bottom
		14–15	54	135	29	
D	½ mile south-west of Stoke Pt	13	36	90	8	
		13	36	90	11	
E	*ca.* ½ mile south-west of small headland just west of Stoke Pt	—	54	135	11	Some gravel patches in vicinity
		—	54	135	8	
F	*ca.* ¾ mile south-west of Gara Pt	10–11	54	135	34	—
		10–11	54	135	29	—
		10–11	54	135	18	—
		10–11	54	135	35	—
		10–11	54	135	29	—
		10–11	54	135	23	—
G	*ca.* 4 cables south-west of Mewstone	14	36	90	30	—
		14	36	90	32	—
		12	30	75	26	—
		12	30	75	35	—
H	*ca.* 3 cables south-west of Mewstone	10–11	54	135	29	—
		10–11	54	135	19	—
		10–11	54	135	37	—
		10–11	54	135	28	—
I	1 mile south-east of Penlee Pt	9–10	54	135	41	Line running west parallel to ridges of rock
		9–10	54	135	43	
		9–10	54	135	7	Line running south cutting across ridges of rock and gullies of muddy gravel
		9–10	54	135	10	
J	Whitsand Bay *ca.* 1¼ miles south of Brawn Rk (between Portwrinkle and Downderry)	10½	54	135	46	Low reefs of rock; *Echinus* slightly smaller than usual (mean diam. of 6–7·7 cm, cf. mean diam. of 8–11·3 cm from marker buoy position)
		10½	54	135	41	
		10½	54	135	75	
		10½	54	135	78	
K	Slightly closer inshore	9	54	135	63	
		9	54	135	52	
		7½–8	54	135	50	





when they are moved from these positions. At the position in Whitsand Bay where large numbers of *Echinus* were counted, all horizontal rock surfaces were almost wholly covered by *Lithothamnion* and another encrusting red alga *Cruoria pellita* (Lyngb.) Fries. Presumably the *Lithothamnion* is either not browsed on heavily or else regenerates more quickly than other species.

At the position near the Tinker Shoal, depth 9 fm, which is near the upper limit for *Echinus* at Plymouth, almost every specimen was partly covered with algal fragments in a similar manner to *Psammechinus miliaris*, though the covering is generally rather more scanty. This form of behaviour is not usually exhibited by *Echinus* in aquaria.

TABLE 2

Type of observation	No. of *Echinus*	Area browsed	Time	Rate of browsing (cm^2/h)
Netting frame on rock, *ca.* 9 fm	6	1 m^2	25 days	2·78
Netting frame on rock, *ca.* 9 fm	3	¼ m^2	11 days	3·2
Surface of upturned boulder, *ca.* 9 fm	1	99 cm^2	22 h	4·5
	1	46 cm^2	22 h	2·1
	1	52 cm^2	18·5 h	2·9
	1	73 cm^2	18·5 h	3·9
Aquarium tank glass. Red algal film	1	161 cm^2	2 days 19 h	2·4
Aquarium tank glass. Green algal film	1	271 cm^2	8 days	1·4

Feeding in aquaria

Echinus will feed readily on the algal film growing on the glass of an aquarium tank. The *Echinus* browses steadily in one spot until after a few hours a small clear area can be observed. This area is slowly enlarged by the urchin working round its periphery leaving remarkably few remnants of the algal film (Plate II). Browsing goes on steadily both during day and night but is not generally continuous for more than a day or two. In one series of observations the rate of browsing for 24 h, apparently continuous browsing, was 5·8 cm^2/h, while for just under 3 days the rate was reduced to 2·4 cm^2/h.

EXPLANATION OF PLATES I AND II

I

Views of the rock surface near Tinker Shoal (depth 9 fm), showing (above) the effect of browsing by *Echinus*, as compared with (below) the state in the absence of *Echinus*.

II

Photographs of the glass front of an aquarium tank showing the results of browsing by *Echinus*. In the upper illustration the *Echinus* is seen in the act of feeding on the thick film of reddish-brown encrusting alga, with its mouth slightly to the left of the centre. The lower illustration shows the clear trail where the algal film has been eaten by an *Echinus*.

Sometimes a second patch on another side of the tank may be browsed for a while with the urchin subsequently feeding alternately on the two distinct areas.

DISCUSSION

The results of the *Echinus* counts show that the urchins are very abundant, particularly below 10 fm. From 10 to 15 fm they may well be considered the dominant species, for in this depth zone the average distribution is one urchin to 4·1 m^2 of rock surface. From the browsing tests (Table 2) it may be seen that 2 cm^2/h is a reasonably conservative rate to assume. Thus if an *Echinus* browses at this rate for four months of the year and at 1 cm^2/h for the remaining 8 colder months it would have covered 1·2 m^2 in the course of the year. On this basis the *Echinus* population is clearing about one-third of the rock surface every year. Naturally the browsed areas would soon be recolonized, but any feeding preferences on the part of the urchins would exercise considerable influence on the proportions of different sessile species. Further predation, possibly rather selective, must be expected from the numerous *Marthasterias glacialis*, occasional *Maia squinado* and from the various species of wrasse.

The distribution of *Echinus* in shallow water or on the shore has attracted much attention in the past. In the north of England and Scotland the species is normally present in quite shallow water—less than 5 fm and in early spring there is a small inshore migration before spawning as far as L.W.S.T. level (Elmhirst, 1922; Stott, 1931). In the south the only records of urchins found on the shore are near Mousehole (Trewavas, 1922) and in the Isles of Scilly (G. M. Spooner, unpublished notes, 1936–7). At Mousehole for the first mile of coast running southwards the 10 fm line lies only 200 yd offshore so that from low water down to 10 fm there is an average gradient of 1 in 10, a situation which is unique for South Devon and Cornwall. It therefore seems very probable that the occasional urchins taken both in this locality and at Scilly are merely strays from deeper water nearby. Reid (1935) postulates a correlation of the intertidal distribution of *Echinus* with the course of the Gulf Stream. This theory will not, however, bear any close examination, for even with the current chart as shown by Reid (for which no reference is given) *Echinus* should be present not only on the shore of north Spain but also along the French coast towards Finistère. But there are no published records of the presence of *Echinus* along the north Spanish coast, and, in a letter from the oceanographic laboratory of Santander, Dr Cuesta writes that it is present but not common. The only two French records, Roscoff and Lanvéoc near Brest (Station biologique de Roscoff, 1951; and Anthony, 1925), are also for places quite close to deeper water. Thus for the whole southern part of its range the species is basically sublittoral and it seems much more reasonable to suppose that a slightly different race is present in the northern part where there is a definite change in behaviour.

SUMMARY

The numbers of *Echinus esculentus* present in known areas have been counted at several positions near Plymouth. A total area of over 5,000 m^2 has been covered, and in this area the average density of *Echinus* is 868 per acre (1–4·7 m^2). A few tests on the rate at which *Echinus* browse on algae and sessile animals have also been made. From these results it has been possible to estimate that where urchins are abundant they are capable of sweeping clear at least one-third of the whole rock surface in the course of a year.

The species is sublittoral for the southern part of its range.

REFERENCES

ANTHONY, R., 1925. Première note sur la faune échinologique littorale de Lanvéoc. *Bull. Soc. zool. Fr.*, T. 50, pp. 319–35.

ELMHIRST, R., 1922. Habits of *Echinus esculentus*. *Nature, Lond.*, Vol. 110, p. 667.

FORSTER, G. R., 1958. Underwater observations on shallow rocky areas in the neighbourhood of Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 473–82.

REID, D. M., 1935. The range of the sea-urchin *Echinus esculentus*. *J. Anim. Ecol.*, Vol. 4, pp. 7–16.

STATION BIOLOGIQUE DE ROSCOFF, 1951. Inventaire de la faune marine de Roscoff: Bryozoaires Echinodermes. *Trav. Sta. biol. Roscoff*, T. 2, Suppl. 4.

STOTT, F. C., 1931. The spawning of *Echinus esculentus* and some changes in gonad composition. *J. exp. Biol.*, Vol. 8, pp. 133–50.

TREWAVAS, E., 1922. Note on the occurrence of *Echinus esculentus* above low tide mark on the Cornish coast. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 833–4.

J. mar. biol. Ass. U.K. (1959) 38, 369–379
Printed in Great Britain

NOTES ON THE SPECIES OF THE PTEROMEDUSAN GENUS *TETRAPLATIA* BUSCH, 1851

By Patricia M. Ralph[1]

Zoology Department, Victoria University of Wellington, New Zealand

(Text-figs. 1–3)

The affinities, structure and distribution of species of the genus *Tetraplatia* Busch, 1851 have been discussed recently by Hand (1955), Beyer (1955) and Rees & White (1957*a*), but ever since these curious bipyramidal medusae (without a typical medusan bell, marginal tentacles, or gastrovascular canals) were described by Busch they have provided much interest for the systematist. There is unanimous agreement that the clearly discernible nematocyst tracts, and the body wall of ectoderm and endoderm separated by a thick, well-defined mesogloea, place them within the Cnidaria as known at present. *Tetraplatia* is usually regarded as the only member of the hydrozoan order Pteromedusae, but Komai (1939) strongly advocates scyphozoan rather than hydrozoan affinities. The purpose of this paper, however, is not to discuss the broad relationships of the Pteromedusae, but to give further evidence that *Tetraplatia chuni* Carlgren, 1909 is a good species. (The structure and affinities of *Tetraplatia* will be discussed in another paper.)

Tetraplatia possesses a body divided into apical and oral regions by an encircling horizontal groove: there are four equidistant radially situated locomotory lappets arising from the groove, two ectodermal (?) statocysts on the undersurface (adoral) of each lappet; the sexes are separate, and there are four endodermal (?) gonads—each gonad has an apical pair of finger-like processes and an oral pair, and the two pairs, are joined by a narrow isthmus in the region of the horizontal groove.

Two species, *T. volitans* Busch 1851 and *T. chuni* Carlgren 1909, are known and described. *T. chuni* lacks flying buttresses between the oral and apical regions of the body and has narrower locomotory lappets than *T. volitans*. Hand's (1955) material from off the Pacific Coast of North America (all of which he recognized as *T. volitans*) showed some specimens in which the buttresses were incomplete or fewer in number than the usual four, and others in which there were three instead of four lappets. Hand also found that, although in general there was little variation in the ratio of the width of the lappet and the space between the lappets (i.e. this space being as wide as the lappet itself), in one or two specimens the space between the lappets was wider, being about twice the width of the lappet, and thus approached the condition described for *T. chuni*. Hand considered that the variation shown by his material demonstrated that *T. chuni* differs little from *T. volitans* and that it may be an aberrant form of it. Rees & White (1957*a*, *b*) working with material from the South Pacific and the North and South Atlantic Oceans recognized *T. chuni* as distinct. They regarded the absence of buttresses in *T. chuni* and the ratio of 1:1 of the length of the apical to oral body regions as a significant and reliable character readily separating *T. chuni* from *T. volitans*. All the material I have examined also demonstrates that *T. chuni* is a good species. There is also evidence that the juveniles of the two species can be as easily recognized as the adults although they have a similar oral to apical body length ratio of 1:1.

---

[1] Nuffield Fellow, 1958.

MATERIAL AND METHODS

Dr F. S. Russell very kindly made available to me 190 specimens of *Tetraplatia volitans* taken by R. V. 'Sarsia' from the Continental Shelf at the approaches to the English Channel and the Bay of Biscay, and transverse and longitudinal serial sections made by Mr A. C. G. Best. The material was collected in the summer months of May to September in the years 1955 to 1957. Ring nets were used in vertical and oblique hauls. The greatest depth fished was approximately 1000 fm but on an average the fishing depth was to about 450 fm. Hauls were made at various times of the day and night but, as collection of *Tetraplatia* was incidental to other tasks and closing nets were not used, correlation is not possible between abundance, diurnal migration and depth, etc. Fifty-seven *Tetraplatia* were taken in nine hauls in 1955, 115 from six hauls in 1956 and 18 from three hauls in 1957. The great majority of the 190 specimens were lightly stained with borax carmine in order to determine sex more readily.

In addition, Dr W. J. Rees and Mr E. White of the British Museum (Natural History) very kindly allowed me to examine all their material of *Tetraplatia* from R.R.S. 'Discovery II' (*T. volitans* and *T. chuni*); 'Carnegie VII'; H.M.S. 'Research' and F.R.V. 'Explorer' (*T. volitans*), as well as transverse sections of a single specimen of *T. volitans* from 'Discovery' collections.

The author wishes to thank Dr F. S. Russell, Director of the Plymouth Marine Laboratory, Dr W. J. Rees and Mr E. White of the British Museum (Natural History) for allowing her to examine material of *Tetraplatia* and for useful advice and encouragement, and to Miss Patricia G. Conway, Exeter, for assistance with measurements of specimens and graphs.

TETRAPLATIA VOLITANS BUSCH

Material collected by R.V. 'Sarsia'

Of the total number of 190 specimens, 173 were available for measurement, the others being damaged or too strongly contracted for accurate measurement. These range in size from 2·1 × 1·4 mm to 9·45 × 2·1 mm, the width being measured at the level of the horizontal groove across the body. The greatest number, 90 in all, were between 4·0 mm and 6·0 mm long and 1·0 mm and 2·5 mm wide. Approximately 80% of the specimens showed a dome-shaped apical tip (Fig. 1, H). The length of the oral region plotted against that of the apical region shows clearly that the length of the oral region is, in specimens above 3·0 mm long, consistently twice that of the apical region and may be more, that is, up to 5:1 (Fig. 2). Juveniles, however, usually have an oral to apical length ratio of 1:1. The ratio for both the juveniles and the adults is similar to that obtained by Hand (1955) and Rees & White (1957*b*).

Oral and apical gonad-lengths, plotted against total body length, show a similar ratio to that of the body regions; the oral lobes are usually the longer in animals above 5·0 mm long. A difference in the length of the oral and apical portions of the gonad has been noted previously. Dantan (1925, Pl. I, fig. 1) figures the oral and apical gonad pairs as approximately equal in length. Beyer's (1955) 'Brategg' specimens showed the oral pairs longer than the apical, particularly in the larger specimens. Beyer thought that the gonad in these large specimens may have been partly spent. The present material, however, indicates that a difference in length of the apical and oral portions of the gonad is probably related to the difference in length of these major body regions and that growth of the gonad in length parallels the growth in length of these regions in the animal as a whole.

Males outnumber females by approximately 5:1 in the present collection. This ratio is based on 100 animals taken over a three-year period in which collections were made by 'Sarsia', and in which the oral region of the gonad is over 0·5 mm in length. Specimens with gonads shorter than 0·5 mm in the oral region were frequently of indeterminate sex.

In the female gonad (Fig. 1, D) the width to length ratio is usually 1:2 but may be 1:3, and in the male gonad (Fig. 1, C) which is narrower and more elongated the ratio is frequently 1:3 and ranges to 1:5. Also, the range of width in the female gonad, 0·21–1·05 mm, is greater than in the male, 0·07–0·49 mm. The two extremes in male gonad width, nevertheless, occur only once, and by far the greatest number have a gonad between 0·21 and 0·28 mm in width.

All sections demonstrate that *T. volitans* has a very narrow waist, from 0·50–0·75 mm in diameter in animals whose total length ranges from 4·5 to 6·5 mm. The width of the waist was measured across the body (excluding the buttresses) in the region of the horizontal groove. One specimen sectioned

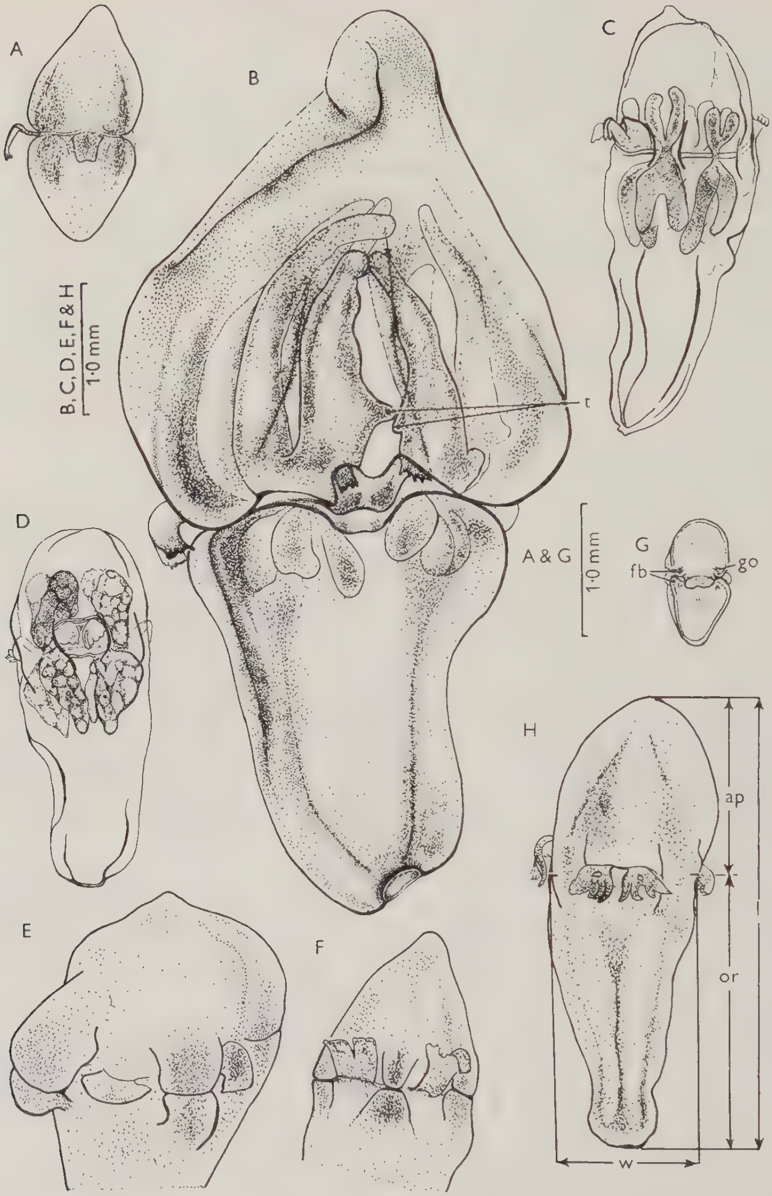


Fig. 1. A and B, *Tetraplatia chuni:* A, juvenile (from N.W. Bouvet Is.); B, adult male (from north of the Antarctic ice edge); C–H, *T. volitans:* C, male, and D, female to show oral and apical paired finger-like portions of the gonad, E–G, specimens from R.R.S. 'Discovery II' collections, E, F, to show the aberrant flying buttresses. G, juvenile to show developing flying buttresses and incipient gonad; H, diagram to show how specimens were measured.

Abbreviations: *ap*, measurement taken for length of the apical region; *fb*, flying buttress; *go*, gonad; *l*, total body length; *or*, measurement taken for length of oral region; *t*, small tubercle on male gonad of *T. chuni*; *w*, width of the body.

longitudinally shows a distinct mesogloeal septum at the equator of two buttresses.

The position of the nematocyst tracts, the nematocysts, and the general external structure of the lappet are as described by Hand (1955). Twenty-five specimens measured for width of the lappet and distance between the lappets showed the usual ratio of 1:1 as known for the species. In no other specimen examined was there any significant departure from this ratio.

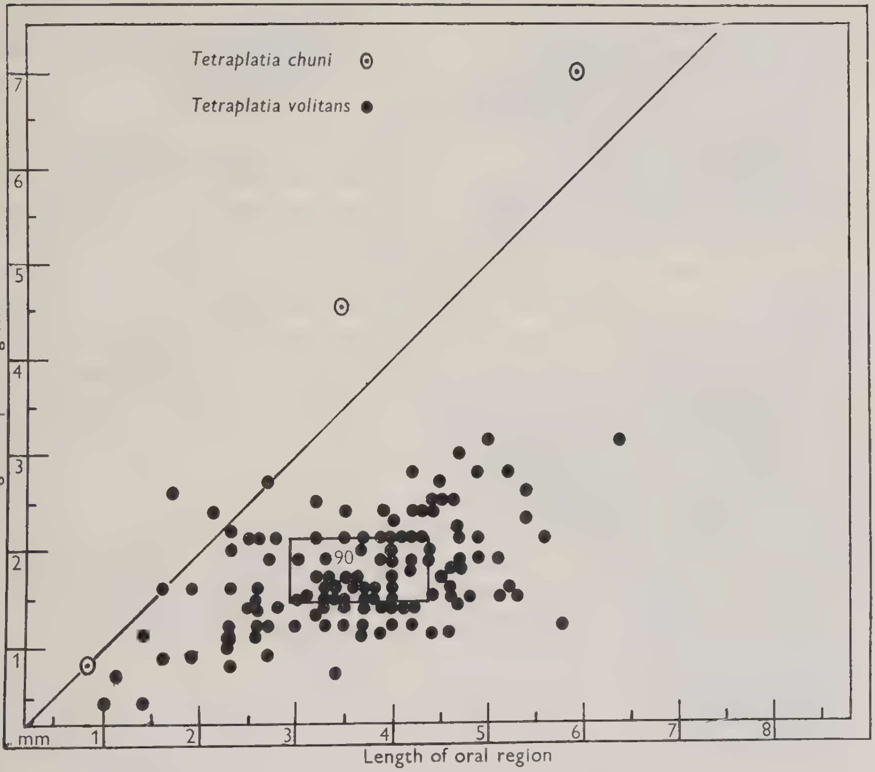


Fig. 2. Graph to show the ratio of the length of the oral region plotted against that of the apical region; line at angle of 45 degrees represents a 1:1 growth ratio of the oral/apical regions; rectangular area represents the ratio of the oral/apical growth length of the greatest number of specimens (90 in all).

Material collected by R.R.S. 'Discovery II'

Specimens examined of 'Discovery' material showed a similar oral: apical length ratio, gonad size and proportions, and ratio of males to females as did those of R.V. 'Sarsia', although gonad structure and sex were not so readily assessed as in the latter. One very young specimen 1·0 mm in total length was noted (Fig. 1, G). This has not been described before; it shows four equidistant swellings at the posterior margin of the oral region and four corresponding swellings opposite them at the anterior margin of the apical region. There seems little doubt that these swellings are incipient buttresses, as there is contact between three of the pairs and in the remaining pair a linking strand of tissue. It is probable that all pairs become fused at this size and that the epithelial layer which is very thin and easily damaged has been torn away. The length ratio of the oral/apical body region is approximately as one is to one, similar to that shown by juvenile specimens in the other material. The width of the lappets and that across the incipient buttresses is approximately equal. Tiny patches of tissue can be seen in the region of the axes of the apical swellings (Fig. 1, G). This is the known region of origin of the gonads and patches of tissue in this region in so young an animal suggests that the reproductive elements develop very early. The presence of swellings in contact to form buttresses and the ratio of 1:1 of the distance between the lappets and the width of the lappets (i.e. the width of the incipient buttresses and lappets is about equal) determine this specimen as *T. volitans*.

Two specimens out of a total of forty-three in the 'Discovery' collection were without four fully developed flying buttresses (Fig. 1, E, F). In each specimen three buttresses appeared incomplete, but contact between oral and apical portions of the buttress is evident and there is an obvious junction. This junction is either a complete mesogloeal septum across the buttress or a thick annulus.

Material collected by 'Carnegie VII'

The two specimens listed by Rees & White (1957*a*) from the Eastern South Pacific Ocean, St. 49/50, 28. xi. 28, show four distinct buttresses and are *T. volitans*. The six small specimens from the Western South Pacific Ocean, St. 93, between Tahiti and Samoa, 31. iii. 29, are not *Tetraplatia*.

Material collected by H.M.S. 'Research'

This material was collected in July, 1900, in the Bay of Biscay at approximately 47° N. 7° W. (Fig. 3).

The two specimens from St. 320 and the single specimen from St. 35*b* recorded by Rees & White (1957*a*) have four fully developed buttresses and are similar to other material taken by R.V. 'Sarsia' of *T. volitans* in the Bay of Biscay. The specimen from St. 33*d* is not *Tetraplatia*.

TETRAPLATIA CHUNI CARLGREN, 1909

Through the courtesy of Dr W. J. Rees and Mr E. White, of the British Museum (Natural History), I was able to examine material collected by R.R.S. 'Discovery II' from high latitudes in the South Atlantic and recognized by these authors as *T. chuni*. One specimen from north-west Bouvet Is. is a juvenile, 1·75 × 0·52 mm, and the other from north of the Antarctic ice edge is a large mature male, 13·0 × 4·0 mm. The ratio of oral to apical body length in both these specimens is figured by Rees & White (1957*b*, fig. 1), and in the present Fig. 2. In *T. chuni*, the oral region is about the same length as, or a little shorter than, the apical. In both specimens of *T. chuni*, the distance between lappets is approximately twice that of the width of the lappet—this is more apparent in the larger one.

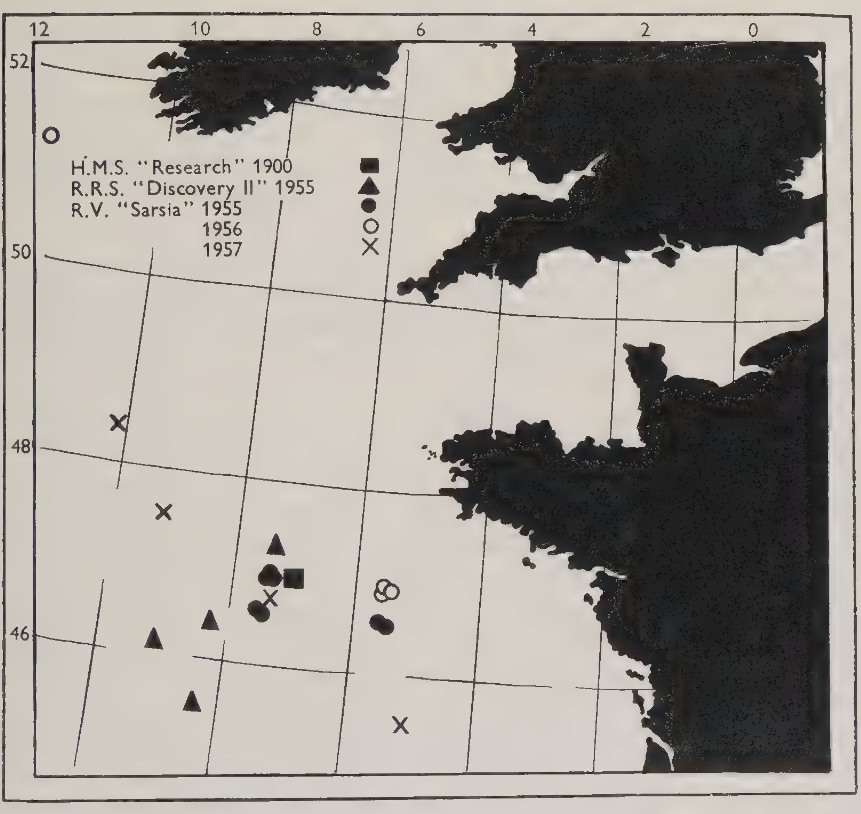


Fig. 3. Catches of *T. volitans* taken in the approaches to the English Channel.

In the large male the gonad is similar in shape (narrow and elongated) to that in *T. volitans*. There is however a distinct, small, outwardly directed tubercle on some of the apical gonad pairs (Fig. 1, B). It has not yet been determined whether these tubercles have any connexion with the exterior by means of an indentation or 'pore' as is known for some material of *T. volitans*, or whether they are in any way involved in the shedding of the gonad products; their tips come very close to the external ectodermal epithelium.

The pairs of finger-like processes of the gonads in the apical region are greater in length (4·5 mm) than in the oral (1·5 mm). As yet, there is insufficient material described to say whether this is always so, or whether it has any relationship to the proportions shown by the major body regions. Carlgren's (1909, Pl. XI, fig. 4) original specimen also had the male gonad in the apical region of greater length than the oral.

REMARKS

Only twenty-three specimens of *T. volitans* have been recorded previously in the area investigated by R.V. 'Sarsia'. They were three specimens in two hauls by H.M.S. 'Research' in 1900, and twenty by R.R.S. 'Discovery II' in five hauls in 1955. All the ships were operating in the summer months. In fact the *Tetraplatia* taken by 'Discovery' and described by Rees & White (1957*a*) must be part of the same population sampled by 'Sarsia' in 1955 (Fig. 3).

Specimens of *T. volitans* from the 'Sarsia' collection demonstrate, as did the twenty specimens of Rees and White from 'Discovery' collections, that in addition to the presence of four flying buttresses, the ratio of the length of the oral to the apical region in adult *T. volitans* is a reliable character for distinguishing this species from *T. chuni*.

Aberrant specimens of *T. volitans* are known in which either some of the buttresses fail to develop, or are partially formed, or only three lappets out of four are present. So far, the numbers examined with particular reference to these features are inadequate to give either a true estimate of the percentage of aberrant individuals likely to be found in a population, or to ascertain if there is any correlation between the percentage of aberrant individuals and a particular locality. Where, however, more than 100 animals from any one area have been examined for these features, the percentage of aberrant individuals has been small. Four of Hand's Pacific Ocean specimens out of a total of 211 were aberrant in these features, but there were none in the present 'Sarsia' collections. The two animals with aberrant buttresses figured in the present paper were taken by R.R.S. 'Discovery II'. The specimens shown in Fig. 1, E, is one out of a total of ten specimens taken off Cape Finisterre, and the other (Fig. 1, F), is one of two specimens from the South Atlantic Ocean. Measurements of Hand's (1955) 'more robust' specimens of *T. volitans* indicate that the width of the lappet may approximate to half the width of the distance between the lappets, i.e. approaching the ratio described for *T. chuni*. However, Hand did not find any in which the lappet was as narrow as that described by Carlgren for *T. chuni* and as shown by the specimens in the present 'Discovery' collection. All the 'Sarsia' specimens had the width of the lappet approximately equal to the length of the distance between the lappets, and were typical of *T. volitans*, irrespective of contraction due to preservation.

While only three specimens of *T. chuni* are known, they all show similar characters whether juvenile or adult. They are without flying buttresses, the oral and apical regions are almost equal in length, and they have narrow lappets, which are only half the width of the distance between the lappets. Juveniles of *T. volitans*, while possessing an oral and apical body region of equal length, have flying buttresses when approximately 1·0 mm in length and lappets in which the width is approximately equal to the distance between them.

All in all, *T. volitans* and *T. chuni* as at present known can be readily recognized and their differing characters summarized as follows. First, and probably still of greatest significance for recognizing the two species, is the presence of four flying buttresses in juvenile and adult of *T. volitans*, and their absence in *T. chuni*. Secondly, the difference in ratio between the major body regions. In *T. volitans* the length of the oral region is much greater than in the apical—a ratio of at least 2:1 and occasionally as much as 5:1 in the adult. In *T. chuni*, on the other hand, oral and apical regions are approximately equal in length, but the apical part may be slightly longer. Thirdly, the width of the lappet in relation to the width of the distance between the lappets is different in the two species. In *T. volitans* the width of the lappet and the distance between the lappets is approximately the same, while in *T. chuni* the distance between the lappets is usually twice the width of the lappet. This relationship of distance between the lappets and the width of the lappet can be seen in both juveniles and adults.

Further, in *T. volitans* the gonad pairs of the oral region are longer than those of the apical part, while in *T. chuni* they are about half the length of the apical. In the former species the difference in length of the gonad pairs in the oral and apical regions seems to be correlated with the difference in length of the major body regions. As yet, there is insufficient evidence to say whether such a relationship is a constant character in *T. chuni*. Three other features are worthy of note: the very narrow waist of *T. volitans* and the much broader waist of *T. chuni*: the dome-shaped apical tip shown by the majority of preserved specimens of *T. volitans* compared with the bluntly pointed apical tip of *T. chuni*; and the greater size of *T. chuni*. Differences in apical shape are without doubt influenced by fixation and preservation, and therefore probably unreliable as a specific character, but the difference in apical shape in *T. volitans* and *T. chuni* may indicate a difference in behaviour and prove to be characteristic of the species. Similarly differences in size may be unreliable as a specific character, particularly in *Tetraplatia* of which *T. chuni* is as yet represented by only two adult specimens. Nevertheless, both these specimens are larger than the majority of adult *T. volitans*; one of them, from north of the Antarctic ice edge, is twice the size.

It seems that *T. volitans* with its four buttresses, larger lappets in relation to body width, and narrower waist, is more highly specialized than *T. chuni*. The sequence of events that led to this higher degree of specialization may have taken place as follows. First, there was the formation of flying buttresses by approximation of oral and apical outgrowths (of all three body-wall layers) and their fusion into a tubular, thick-walled strut between oral and apical regions of the body; the presence in the very tiny juvenile animal of the approximating outgrowths and the partial or complete septum of mesogloea in a few adult animals support this theory of the origin of the buttresses. At the moment, therefore, I cannot agree with Carlgren or Beyer (1955, p. 112) that the lack of buttresses in *T. chuni* is due to their degeneration from an originally buttressed condition. This conclusion is supported by the absence of buttresses in the juvenile *T. chuni*. Nor can I agree with Hand (1955, p. 337) that the sole function of the buttresses is to increase the digestive area, although this increase is incidental to the formation of buttresses. Their structure and shape make it reasonable to suppose that their primary function is now to afford some support for the body, even if, as indicated by the juvenile in the 'Discovery' collections, they originated as outgrowths from the gastric cavity. On the assumption that the buttresses give some support, a narrowing of the waist is possible and this would bring the lappets more closely into the groove between the buttresses, so that in effect the body becomes more streamlined. The neat fit of the lappets between the buttresses must also make for efficient swimming, as most, if not all of the locomotory beat would be effective, there being little or no lateral diversion of water. Increased growth of the oral body region may have arisen from increased swimming efficiency and the necessity for a highly mobile mouth as an efficient food catching mechanism. *Tetraplatia chuni*, on the other hand, lacking buttresses and having a long groove between narrow lappets, is probably a much less efficient swimmer than *T. volitans*. As yet, we may not have fished deeply enough to catch numbers of *T. chuni*.

SUMMARY

Collections of *Tetraplatia* made by R.V. 'Sarsia' from 1955 to 1957 are described and discussed in relation to specimens taken by R.R.S. 'Discovery II'; 'Carnegie VII'; H.M.S. 'Research' and F.R.V. 'Explorer'.

The results confirm that *T. volitans* Busch, 1951, and *T. chuni* Carlgren, 1909, are separate species which can be easily separated both in the juvenile and adult stages.

The difference in length of the gonad in the apical and oral regions in *T. volitans* is found to be correlated with the difference in length of these two major body regions. A similar gonad-length to body-length relationship can be seen in *T. chuni*, but insufficient material has been captured to ascertain whether this is a constant character in this species.

In general males occur in greater numbers than females in *T. volitans*. The known mature specimens of *T. chuni* are both males.

T. volitans is regarded as more highly specialized than *T. chuni*.

REFERENCES

BEYER, F., 1955. A new record of *Tetraplatia* (Pteromedusae). *Nytt Mag. Zool. Oslo*, Vol. 3, pp. 106–12.

BUSCH, W., 1851. *Beobachtungen über Anatomie und Entwicklung einiger wirbellosen Seetiere.* 143 pp. Berlin.

CARLGREN, O., 1909. Die Tetraplatien. *Wiss. Ergebn. 'Valdivia'*, Bd. 19, Lief. 3, pp. 75–122.

DANTAN, J. L., 1925. Contribution à l'étude du *Tetraplatia volitans*. *Ann. Inst. océanogr. Monaco*, N.S. II, Fasc. 5, pp. 429–59.

HAND, C., 1955. A study of the structure, affinities and distribution of *Tetraplatia volitans* Busch (Coelenterata: Hydrozoa: Pteromedusae). *Pacific Sci.*, Vol. 9, pp. 332–48.

KOMAI, T., 1939. On the enigmatic Coelenterate *Tetraplatia*. *Jap. J. Zool.*, Vol. 8, pp. 231–50.

REES, W. J. & WHITE, E., 1957*a*. New observations on the aberrant medusa *Tetraplatia volitans* Busch. *'Discovery' Rep.*, Vol. 29, pp. 129–38.

REES, W. J. & WHITE, E., 1957*b*. Two new records of *Tetraplatia chuni* Carlgren from the South Atlantic. *J. Fac. Sci. Hokkaido Univ.*, Ser. 6 (Zool.), Vol. 13, Nos. 1–4, pp. 101–4.

J. mar. biol. Ass. U.K. (1959) 38, 381–394
Printed in Great Britain

ON THE SEXUAL BIOLOGY OF *PANDALUS BOREALIS* (CRUSTACEA DECAPODA)

I. HISTOLOGY OF INCRETORY ELEMENTS

By D. B. Carlisle

The Plymouth Laboratory

(Text-figs. 1–16)

Anatomical and histological details are here given of two organ systems, which, my experiments lead me to believe, may secrete hormones concerned in the sex reversal that overtakes all male *Pandalus borealis* Krøyer. The descriptions eschew any attempt at detailed cytology, and refer exclusively to prawns taken from the population of Gullmarfjord in south-west Sweden. There is reason to believe that, as with *Palaemon* (= *Leander*) *serratus* (cf. Carlisle, 1955), different populations may vary widely in the detailed topography of the endocrine organs, as well as in appearance, life history and growth rate (cf. Horsted & Smidt, 1956).

The organ systems to be described are the X organ-sinus gland complex of the eye-stalk and the vas deferens gland system or *glande androgène*.

A note is necessary on the orientation of organs within the eye-stalk. When a prawn is at rest the eye-stalks are held outwards. In this position the eyes themselves are lateral. When a prawn is lifted out of the water, or when it is looking forward, the eye-stalks are turned forward and (in the former situation) are held in close against the head, so that the eyes hold an anterior position. This mobility of the eye-stalk, one of the characteristic features of the stalk-eyed Crustacea, makes the use of the terms anterior and posterior confusing. Following a suggestion made by Dr F. S. Russell, therefore, I propose using the terms abaxial and adaxial, with the same sense as their usage in descriptions of medusae. Under this terminology the eye will be referred to as distal (never as anterior); the side of the eye-stalk which is towards the axis of the body, i.e. the side which is medial when the eye-stalks are held in close or which lie anteriorly when the eye-stalks are turned outwards, will be called the adaxial side; the opposite side, i.e. the lateral side when the eye-stalks are held close, or the posterior side when they are turned outwards, will be called the abaxial side.

THE X ORGAN-SINUS GLAND COMPLEX

In decapods the sensory papilla has been reduced to a sensory pore, or, in many species, lost. The pore is retained in *P. borealis* and this is the only point where the X organ-sinus gland complex approaches the superficies of the eye-stalk. The gross internal anatomy of the eye-stalk differs little from that which has been reported as normal in decapods. The eyes are large as befits a deep-water animal, and the eye-stalks are relatively short and stubby, though highly mobile.

The main nervous nuclei present in the eye-stalk are the medulla terminalis (perhaps more correctly, though less usually, called the lobus terminalis) of the protocerebrum, and the medullae interna et externa. The last is succeeded by the lamina ganglionaris, the most proximal layer of the retina. The neurosecretory cells are concentrated into three major groups, one in each of the medullae. They may, following the terminology of Knowles & Carlisle (1956), be called the medulla terminalis ganglionic X organ (*m.t.g.x.*), the medulla interna ganglionic X organ (*m.i.g.x.*) and the medulla externa ganglionic X organ (*m.e.g.x.*), respectively (see Figs. 1–8). From these organs neurosecretory fibres run more or less directly to the sinus gland, which lies dorso-adaxially against the medulla interna. The tracts of fibres unite before entering the sinus gland and are joined by a relatively minor group of fibres coming from the brain; the four tracts thus enter together and the gland is not divided into separate lobes as in *Palaemon* (see Carlisle & Knowles, 1959). A further tract

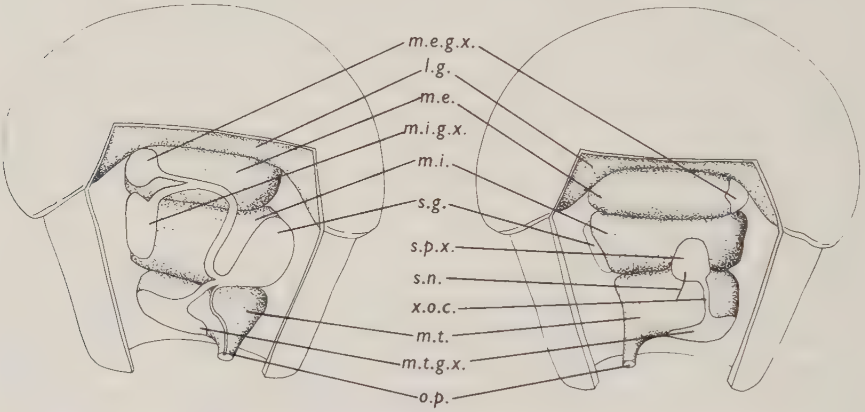


Fig. 1. Dorsal (left) and ventral (right) dissections of a left eye-stalk of *Pandalus borealis*, with all the non-nervous tissue omitted.

Lettering guide to Figs. 1–8. *d.p.s.*, dorsal pigment spot; *l.g.*, lamina ganglionaris; *m.e.*, medulla externa; *m.e.g.x.*, medulla externa ganglionic X organ; *m.i.*, medulla interna; *m.i.g.x.*, medulla interna ganglionic X organ; *m.t.*, medulla terminalis; *m.t.g.x.*, medulla terminalis ganglionic X organ; *o.p.*, optic lobe peduncle; *s.g.*, sinus gland; *s.n.*, sensory nerve of sensory pore; *s.p.*, sensory pore; *s.p.x.*, sensory papilla X organ; *x.o.c.*, X organ connective.

of neurosecretory fibres, the X organ connective, runs from the *m.t.g.x.* to the sensory papilla X organ (*s.p.x.*), which is attached ventrally to the medulla terminalis and projects like a finger into the ventral blood sinus of the eye-stalk, bathed on all sides by blood (see Figs. 2, 6–7), until at its distal end it attaches to the sensory pore. This, a thinning of the cuticle, ventrally on the eye-stalk near the limit of the eye, is lined by a continuous layer of sensory nerve cells, whose axons form a nerve, distinct from the X organ connective, running to the medulla terminalis (see Figs. 1, 2).

The ganglionic X organs

Each of these groups of neurosecretory cells consists of about 30 cells compactly arranged into a discrete organ with no intermixture of normal neurones; nor are there any isolated neurosecretory cells scattered among the association areas, such as are to be found in *Lysmata* (see Carlisle, 1953*c*). The cells are about $75 \times 45\mu$, densely granulated with granules about at the limit of resolution of a ×45 objective (0·4–0·5 μ). The granules have the same staining properties as in other decapods (see Carlisle & Knowles, 1959). In my preparations the cells are roughly rhomboidal in shape, with the axon forming the continuation of one end of the long diagonal (see Fig. 9). The nucleus is about 20–22 μ in diameter and in most cells contains three well-defined nucleoli.

In the *m.t.g.x.* the more adaxial cells send fibres to the sinus gland while the more abaxial ones direct their fibres to the *s.p.x.* About two thirds of the cells fall into the former and one third into the latter category. The axons of the adaxial cells leave the *m.t.g.x.* on the surface of the medulla terminalis at the adaxial corner of the X organ, and run along the surface of the medulla distally to the sinus gland, following the shortest route along the surface of the ganglionic chain. The fibres from the abaxial cells also leave on the surface of the medulla, forming the X organ connective, and run directly along the ventral surface distally to the *s.p.x.*, again following the shortest route (see Figs. 1, 2).

The axons of the *m.i.g.x.* all leave together at the distal corner of the X organ on the surface of the medulla interna. The tract runs distally on to the dorsal surface of the medulla externa, where it is joined by that from the *m.e.g.x.*, turns in a broad bend back on to the medulla interna and, first making another bend, joins with the tract from the *m.t.g.x.* which runs to the sinus gland. This tract, then, makes a broad S-bend on the dorsal surface of the medullae interna et externa.

All the tracts are on the surface of the ganglionic chain and are readily visible in the fresh dissection as opaque bluish white lines. In some few dissections there appears to be a nerve linking the *s.p.x.* with the sinus gland via the dorsal pigment spot. I am uncertain of this.

The cytology of the neurosecretory cells is in no way remarkable. Using Potter's methods, however (Potter, 1954, 1958), it was possible to distinguish

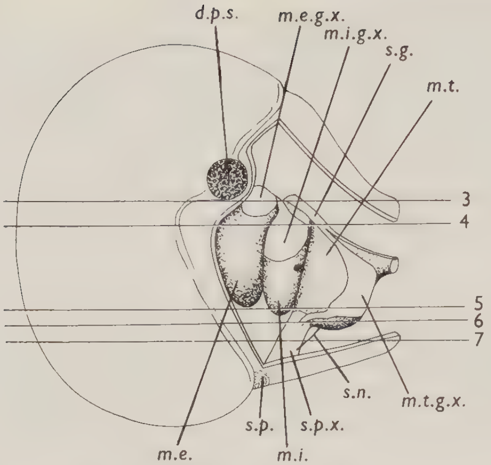


Fig. 2. Abaxial dissection of a left eye-stalk, with all the non-nervous tissue omitted. The numbered lines indicate the approximate planes of the sections illustrated in the like-numbered figures. For lettering guide see Fig. 1.

▤ Sinus gland
▦ Sensory pore X organ
▩ Ganglionic X organs
▨ Muscle
▥ Retroretinal tissue
░ Blood

Key to figs. 3–8

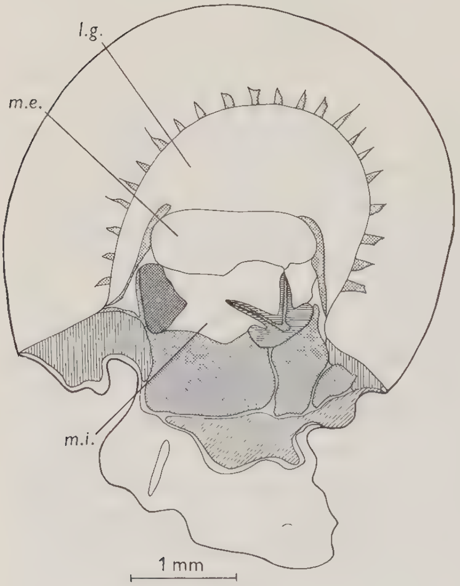


Fig. 3

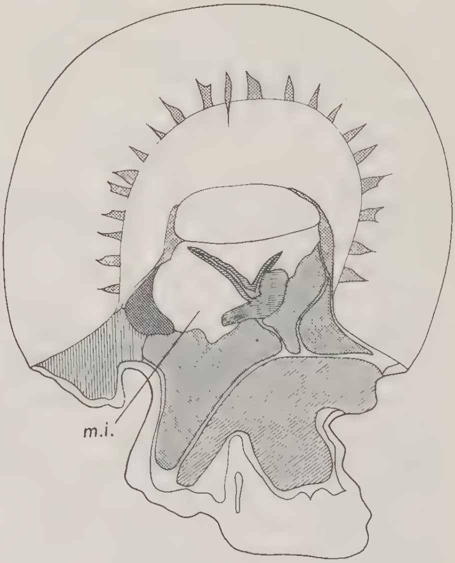


Fig. 4

Figs. 3–7. Horizontal sections through a single left eye-stalk taken in June at the levels indicated in Fig. 2. The abaxial side is to the left. The lettering guide is given below Fig. 1.

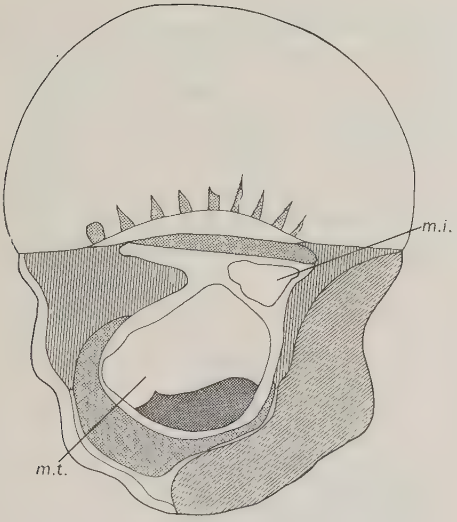


Fig. 5

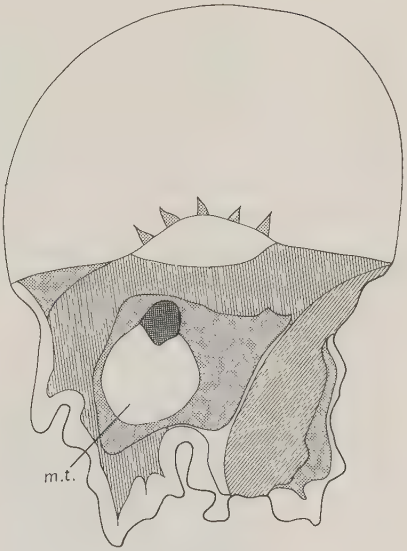


Fig. 6

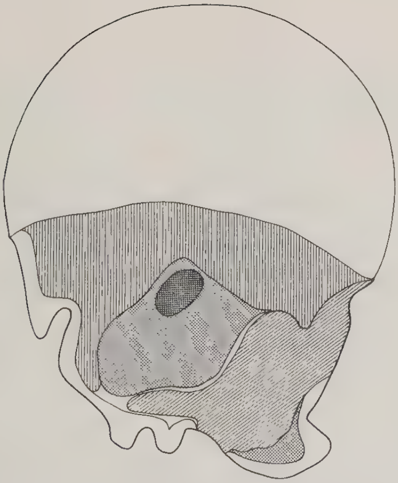


Fig. 7

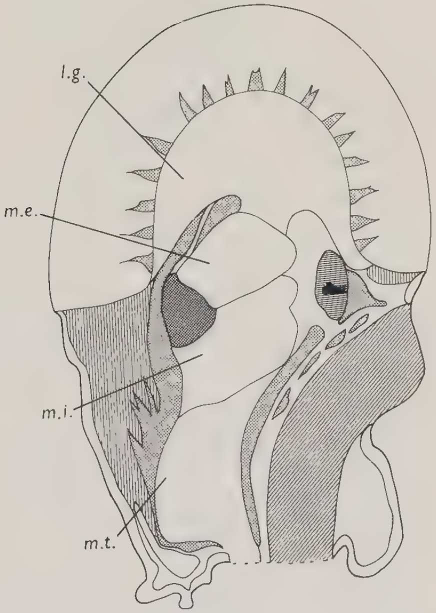


Fig. 8

Figs. 5–7. See legend to Fig. 3 opposite.

Fig. 8. An approximately horizontal, somewhat oblique section of a left eye-stalk. The adaxial side is to the left of the figure. Note particularly the position of the type IV nerve endings indicated in black in the sinus gland. The patch of retroretinal tissue to the right of the eye-stalk retains the acinar structure, although this animal was killed in June. The lettering guide is given below Fig. 1.

by their staining properties five types of cells in the three groups. The *m.i.g.x.* cells were uniform in staining yellow with orange G and constitute the first type. The secretion of those cells of the *m.t.g.x.* which supply the *s.p.x.* stains red with azocarmine; these cells constitute type II. The majority of the cells in the *m.t.g.x.* stain bluish purple with azocarmine and aniline blue (type III), while cells observed only in non-sexual and female animals take up osmium tetroxide strongly and stain blue with aniline blue (type IV). The cells of the *m.e.g.x.* constitute type V and contain, like type II cells, a secretion which stains strongly red with azocarmine. The cells of types I–IV all contain the characteristic 'mulberries', so well-known in crustacean (and other) neurosecretory cells. These are agglomerations of the smaller secretory granules into masses about 3–4 μ across, having the appearance of mulberries (see Fig. 9 A). These mulberries are absent from the type V cells, which instead are densely vacuolated, with spherical vacuoles of all sizes from the limit of resolution up to 5 μ across, squeezed in among the red staining granules in an azan preparation (see Fig. 9 B).

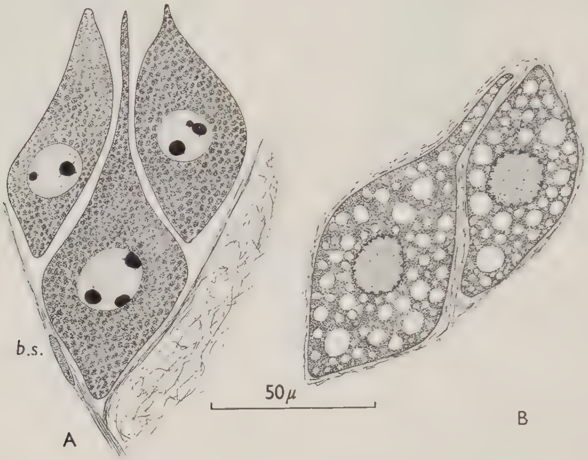


Fig. 9. Neurosecretory cells from the eye-stalk. A. Type I cells from the medulla interna ganglionic X organ. B. Type V cells from the medulla externa ganglionic X organ. *b.s.*, blood sinus.

The sinus gland

Like the neurosecretory cell groups which supply it, the sinus gland may be shown to consist of at least four kinds of elements of differing tinctorial affinities. The staining properties, however, do not correspond exactly to those of the cells and it seems likely that, as in *Palaemon* (Carlisle, 1958), the secretory material undergoes a physico-chemical change as it passes down the axons. Those nerve endings which are strongly osmophilic are stained red by azocarmine. Tracing of individual fibres shows that these endings derive from cells which are likewise osmophilic, but which stain blue with aniline blue (type IV cells). These endings are rather closely circumscribed in position (see Fig. 8). The centre of the gland is occupied by two intermingled types of endings, one staining blue with aniline blue and the other reddish mauve with azocarmine and aniline blue. The blue endings derive from type III cells, while the reddish mauve ones appear to correspond to type V cells. These latter endings are also found scattered throughout the rest of the gland among the fourth type of endings, which stain red with azocarmine and come from the type I cells of the *m.i.g.x.* The sinus gland consists almost entirely of these nerve endings of varying tinctorial affinities.

The body of the gland in a large female forms a perforated disc about 500 μ in diameter and 250 μ thick. Not more than about 25 nuclei of the connective tissue supporting cells may be seen in it and this tissue forms a minor fraction of the total tissue of the gland. As always, the gland surrounds the point where the blood vessels from the interior of the eye-stalk emerge into the outer blood sinus of the eye-stalk. It is usually stated that these blood vessels run from the inner blood sinus of the ganglionic chain, but in *Pandalus*, as in other Natantia that I have examined, there is no such inner blood sinus. The blood supply of the ganglionic chain consists of a number of small vessels which unite into two main vessels, running from the medulla interna and the medulla externa respectively to unite within the sinus gland before debouching into the outer blood sinus. As may be seen from Figs. 3 and 4, sinus gland tissue surrounds the outer part of these two blood vessels and the body of the gland is arranged around the opening into the blood sinus.

The tract of fibres from the various neurosecretory centres enters the sinus gland by the proximo-abaxial corner (see Fig. 4). The fibres immediately split up and the numerous club-shaped endings of each fibre run perpendicularly to the surface of the gland to abut on to the blood sinus or on to one of the blood vessels.

The sensory papilla X organ

Since the sensory papilla has been reduced to a sensory pore lying close against the border of the eye, this organ has come to lie internally. In *P. borealis* the cuticle is almost as thick over the sensory pore as elsewhere on the eye-stalk so that the term becomes something of a misnomer (see Fig. 12). The bulk of the organ is made up of neurosecretory axons and nerve endings, together with a rather large amount of supporting connective tissue and a groundwork of epithelium-like cells. The cytoplasm of these cells will not take up any of the common stains. About 8–10 neurosecretory fibres run into the *s.p.x.* and divide several times before terminating in the characteristic 'onion bodies' of the *s.p.x.* Each branch terminates in one of these bodies, which in section looks much like a section of an onion; the onion bodies corresponding to all the branches of any one axon lie together and are bound into a group by a fibrous membrane. They stain with aniline blue in Heidenhain's azan technique. The whole organ is bounded on all sides, except where attached to the medulla terminalis or to the sensory pore, by a connective tissue sheath. It is cylindrical and abuts into a blood sinus (see Figs 2, 6 and 7). A few small blood vessels penetrate into its tissue. The sensory nerve cells which line the sensory pore are in no way remarkable, having scant cytoplasm and fine axons. The staining of the nuclei suggests that there may be two types, one about three times as abundant as the other (see Fig. 12).

There is a very noticeable seasonal variation in females in the histology of the *s.p.x.* (cf. that in *P. kessleri*, Aoto & Nishida, 1956). In April–June, a period between breeding seasons, when the eggs have recently hatched and the ovary has not yet begun to grow for the next breeding season, the epithelioid cells of the *s.p.x.* appear to lose the cell boundaries and to become syncytial; there is little trace of secretory material in the onion bodies (Fig. 10). In September, when the ovaries have begun vitellogenesis for the forthcoming oviposition in December, the epithelioid cells are discrete, and there is much secretory material in the onion bodies and around them. The space between the onion bodies and the surrounding fibrous sheath may be grossly distended with colloid (hyaline or loaded with secretory granules) so that the sheath surrounds a space ten times the volume of the contained group of onion bodies (Fig. 11). The younger males show a condition approximating to that of the April females; the males approaching sex reversal a condition similar to that of the September females. This is true of the males at all times of the year; there is no obvious seasonal variation in the male *s.p.x.*

THE RETRORETINAL ORGAN

The retroretinal organ of the eye-stalk lies behind the lamina ganglionaris of the retina. Like the *s.p.x.* it shows strong seasonal variation. The structure is the same in both males and females and the seasonal cycles are in step in the two sexes. It does not, therefore, vary with the state of the *s.p.x.*, which has no seasonal variation in the male. At certain seasons of the year, notably September, it has the appearance of a secretory organ, but at other times it has the appearance more of adipose tissue. It does not seem to vary with the stage of the moult cycle.

In a specimen taken in September the retroretinal organ has the appearance roughly of a portion of the exocrine tissue of a mammalian pancreas, with circular nests of cells (acini) surrounding a central lumen (Fig. 13). The cells, which are columnar, are loaded with granules. Little material is visible in the lumen of the acini. These acini fill any available space behind the retina; their distribution varies widely in different individuals and seems

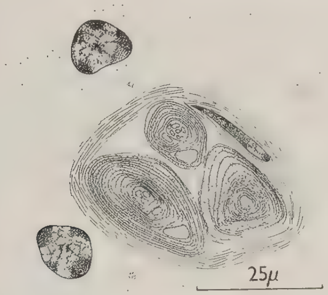


Fig. 10. A single group of onion bodies embedded in a syncytium of epithelioid tissue from the sensory papilla X organ of the eye-stalk drawn in Figs. 2–6, a specimen taken in June.

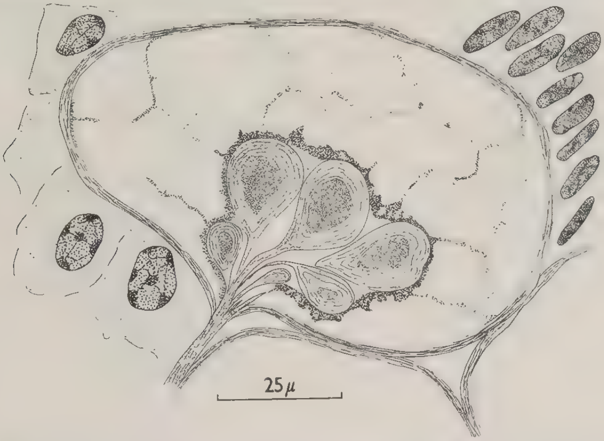


Fig. 11. A single group of onion bodies from a sensory papilla X organ of a specimen taken in September. In this section the branching of the axon into the various branches, each terminating in an onion body, is clearly seen. The onion bodies are full of secretion and the sheath is grossly distended into a cyst. Portions of other cysts are seen by the lower right corner, the discrete epithelioid cells to the left, and the nuclei of the sensory neurones of the sensory pore by the top right corner.

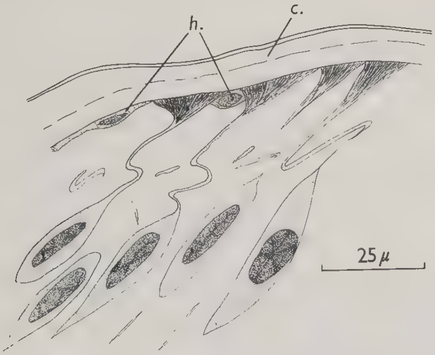


Fig. 12. Sensory cells of the sensory pore, from the same specimen as Fig. 9. Note the relative thickness of the chitinous cuticle over the pore and to one side. *c.*, chitinous cuticle; *h.*, hypodermal cells.

to depend on the space available (see Figs. 3–8). In April the acinar structure has broken down to a great extent and the cells have become much swollen with stored products, giving something of the appearance of a mammalian adipose tissue (Fig. 14). Sometimes a few small nests of acinar structure may be seen to one side of the eye-stalk, but these can be seen to grade evenly into the remaining 'storage tissue' (cf. Fig. 8). At this time of year the tissue occupies more space.

Whatever the function of this tissue, I can find no evidence (as I hope to report in detail in a later paper) that it participates in controlling the sexual cycles of *Pandalus*. Nor do I believe it to be an endocrine organ at all. Indeed, so unlike an endocrine organ is it in appearance that I should have made no mention of it if it had not seemed likely that it is homologous with the organ termed the Y gland by Aoto & Nishida (1954) and termed the circum-orbital gland by Aoto & Nishida (1956). (Each of these names is inappropriate since the name 'Y organ' had already been used for an endocrine gland in Crustacea by Gabe, 1953, while it is by no means around the orbit, but in the eye-stalk, well away from the orbit). Aoto & Nishida (1956) concluded that their 'circum-orbital gland' was incretory in nature and acted antagonistically to the X organ in controlling the breeding cycle in *P. kessleri*. The retroretinal organ, although it shows cycles of activity which, correlated with the annual cycle, must perforce be correlated with the annual breeding cycle, nevertheless must be regarded as controlled by rather than controlling the annual cycle. In neither appearance nor physiological properties (as I hope to show in a later paper) can it be likened to an endocrine organ.

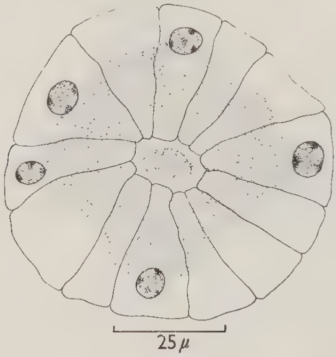


Fig. 13

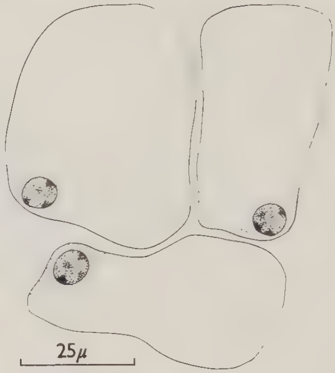


Fig. 14

Fig. 13. An acinus of the retroretinal organ from a specimen taken in September.

Fig. 14. Retroretinal tissue from the eye-stalk of a specimen taken in June.

THE VAS DEFERENS GLAND

This gland lies in and on the wall of the vas deferens and was first described in *Orchestia* by Charniaux-Cotton (1954), who at first gave it no name. Knowles & Carlisle (1956) called it the vas deferens gland, while the original discoverer (1956) later named it *la glande androgène*. The former name seems preferable, for, by common anatomical convention, it is customary to name an organ from its appearance or topography and not from its supposed function; for even if the function is correctly induced this may not be the only or the main function of the organ.

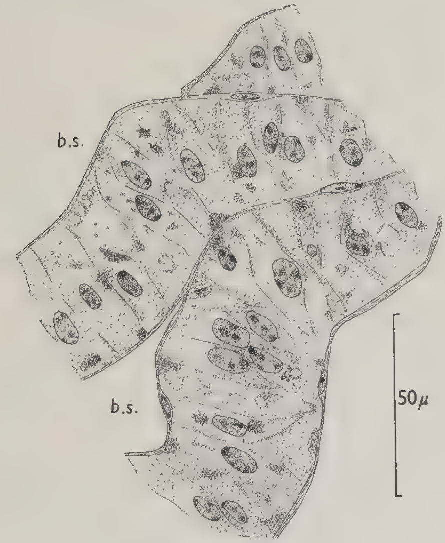


Fig. 15

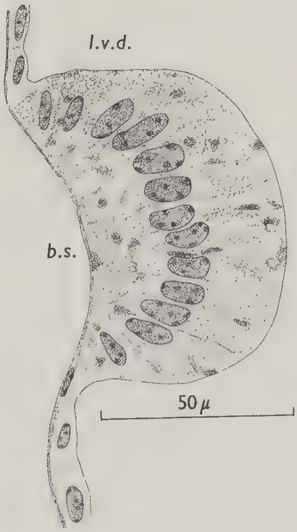


Fig. 16

Fig. 15. Part of the massive portion of the vas deferens gland. Note the fibrous sheath. *b.s.*, blood sinus.

Fig. 16. A transverse section of the strand of the vas deferens, with a portion of the squamous epithelium of the duct. Note the continuous fibrous sheath over the outer side of both duct and strand. *b.s.*, blood sinus; *l.v.d.*, lumen of the vas deferens.

In a fully functional male *P. borealis*, some few moults before sex reversal, the gland is well developed. The bulk of the organ is attached to the distal end of the vas deferens, near where it swells as a vesicula seminalis. It consists of two or more cords of cells which entwine round one another making an irregular heap (Fig. 15). The cells are columnar, about 45 × 10 μ, with nuclei about 10 × 7 μ. The cytoplasm is granulated and frequently (though not always) vacuolated. The general structure is of entwining strips of columnar epithelium, covered by a tenuous, though definite, connective tissue sheath. Close inspection shows that this massive organ (about 250 × 100 μ) is attached to a strip of specialized epithelium of the vas deferens. In serial section this strand of cells, of the same nature as those of the bulk of the vas deferens gland, is seen to run the whole length of the vas deferens along one side. Most of my series of sections are disorientated but I believe that this strand runs along the median edge of the vas deferens. The epithelium covering the vas deferens is a single-layered pavement epithelium with much flattened nuclei covered by a thin connective tissue sheath which burgeons into a muscular sheath over the distal portion of the duct. Along the strand the cells are columnar with the tallest in the centre. The strand is about 10–15 cells wide, convex towards the cavity of the vas deferens, concave towards the surrounding blood sinus (Fig. 16). At intervals along it there twine out cords of cells into the blood sinus. More distally these are bigger, culminating in the most distal massive vas deferens gland. I believe that the whole structure—strand, minor cords of cells and the major massive group—form together the vas deferens gland complex. Certainly the massive part may be shown to have the same function as the gland of *Orchestia*, while the rest is closely connected with it.

In male *P. borealis*, which are of the size at which sex reversal takes place, the vas deferens gland is reduced to nothing more than the strand along the length of the vas deferens, with distally no more than 10–20 cells of cuboidal shape representing the massive part of the gland. This may apply even if the vas deferens is still full of sperm. In animals of the first non-sexual intermoult (after the moult at which the male characters are lost) the vas deferens remains, but even the strand of the vas deferens gland disappears. Sometimes a much-diminished strand with pycnotic nuclei may be distinguished. In the next intermoult the vas deferens itself is gone. The testis may persist for several moults after the animal has become fully functional as a female, but eventually it too degenerates leaving no trace.

DISCUSSION

The X organ-sinus gland complex of *P. borealis* is of the type which seems to be primitive in decapods and somewhat similar to that found in *Lysmata* (Carlisle, 1953*c*). It differs from that in *Palaemon* chiefly in that the latter has lost the sensory pore. One secondary feature of the system in *Pandalus* appears to be the shortening of the X organ-sinus gland tract, which seems primitively to run in an S-shaped curve (or more complicated convolutions) from the *m.t.g.x.* to the sinus gland (see Carlisle & Knowles, 1959). The complications of the course of this tract are presumably a consequence of the distortion of the topography of this region by the development and elongation of the eye-stalk. The straight route of this nerve tract in *Pandalus* is thus presumably secondary.

The vas deferens gland is not yet sufficiently well known in many species to enable one to say whether the condition of this gland in *P. borealis* is exceptional. In Mme Charniaux-Cotton's description and figures of the gland in *Orchestia*, where it was first described (Charniaux-Cotton, 1956), the gland is seen to lie entirely outside the muscle and connective tissue sheath of the vas deferens. This I also find to be the condition in *Processa canaliculata* and *Palaemon serratus*, in both of which the sheath is continuous between the vas deferens and the gland. In four species of Pandalidae, however, the sheath runs outside the gland, which is nowhere delimited from the vas deferens, but forms indeed, in places, part of the wall of the duct (see Fig. 14). Only when sex reversal is imminent does the gland become detached from the vas deferens, and the muscular sheath pass between them. Which of these conditions is primitive and which is the more widely distributed we have as yet no means of knowing. One species which would most repay study in this connexion is *Lysmata seticaudata*, the only non-pandaloid natatian which is known to be a protandric hermaphrodite. It may be that the condition found in *Pandalus* is bound up with protandric hermaphroditism.

A cursory examination which I have made of two other species of *Pandalus* —*P. montagui* Leach and *P. bonnieri* Caullery—and of the related *Pandalina brevirostris* (Rathke), revealed but minor variations in the two incretory systems from the conditions described here for *P. borealis*. It seems likely, then, that it represents the norm for the genus. The two organ systems which I have described—the X organ-sinus gland complex and the vas deferens gland system—are known in other species of crustaceans to act as endocrine organs affecting or, in part, controlling the sexual biology. I hope to show in later papers that they have this function also in *P. borealis*. In this histological investigation it has become abundantly clear that the structure of these two systems gives one every ground for ascribing an incretory activity, and, indeed, in so far as secretion is a histological concept, these organs are, by definition, organs of internal secretion.

I should like to acknowledge the friendly assistance afforded me in this work by the former director, Dr G. Gustafson, and the staff of K. Svenska Vetenskapsakadamien Kristinebergs Zoologiska Station, and to thank Mr A. C. G. Best, who made many of the preparations upon which this account is based.

SUMMARY

The X organ-sinus gland complex of *Pandalus borealis* illustrates a condition which is relatively primitive in decapods. The complex is described and the histology and topography of the various parts figured. The only major departure from the primitive condition is the secondary shortening of the X organ-sinus gland neurosecretory tract. The annual cycle of activity in the sensory papilla X organ is briefly described and discussed. The vas deferens

gland (*glande androgène*) is described and its special features noted. The correlation between its condition and the sexual state of this protandric hermaphrodite is stressed.

REFERENCES

AOTO, T. & NISHIDA, H., 1954. (Quoted by Aoto & Nishida, 1956.)

—— 1956. Effect of removal of the eyestalks on the growth and maturation of the oocytes in a hermaphrodite prawn, *Pandalus kessleri*. *J. Fac. Sci., Hokkaido Univ.*, Ser. 6 (Zool.), Vol. 12, pp. 412–24.

CARLISLE, D. B., 1953*a*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). IV. On the site of origin of the moult-accelerating principle—experimental evidence. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 285–92.

—— 1953*b*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). V. The ovarian inhibiting hormone and the hormonal inhibition of sex reversal. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 355–72.

—— 1953*c*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). VI. Notes on the structure of the neurosecretory system of the eyestalk. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 435–47.

—— 1955. Local variations in the colour pattern of the prawn *Leander serratus* (Pennant). *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 559–63.

—— 1958. Activation of hormonal secretions: a crustacean chromactivator. *Nature, Lond.*, Vol. 182, pp. 32–4.

CARLISLE, D. B. & KNOWLES, F. G. W., 1959. *Endocrine Control in Crustaceans.* Cambridge University Press.

CHARNIAUX-COTTON, H., 1954. Découverte chez un Crustacé amphipode (*Orchestia gammarella*) d'une glande endocrine résponsable de la différenciation des caractères sexuels primaires et sécondaires mâles. *C.R. Acad Sci., Paris*, T. 239, pp. 780–2.

—— 1956. Determinisme hormonal de la différenciation sexuelle chez les Crustacés. *Ann. biol.*, T. 32, pp. 372–99.

GABE, M., 1953. Sur l'existence chez quelques Crustacés malacostracés, d'un organe comparable à la glande de la mue des Insectes. *C.R. Acad. Sci., Paris*, Vol. 237, pp. 1111–12.

HORSTED, S. A. & SMIDT, E., 1956. The deep sea prawn (*Pandalus borealis* Kr) in Greenland waters. *Medd. Komm. Havundersøg., Kbh.*, N.S., Bd. 1, No. 11, 118 pp.

KNOWLES, F. G. W. & CARLISLE, D. B., 1956. Hormonal control in the Crustacea. *Biol. Rev.*, Vol. 31, pp. 396–473.

POTTER, D. D., 1954. Histology of the neurosecretory system of the blue crab *Callinectes sapidus*. *Anat. Rec.*, Vol. 120, p. 716.

—— 1958. Observations on the neurosecretory system of portunid crabs. *Proc. 2nd int. Symp. Neurosecretion, Lund*, 1957. Berlin: Springer.

J. mar. biol. Ass. U.K. (1959) 38, 395–406
Printed in Great Britain

THE SPAWNING OF *ARENICOLA MARINA* (L).

I. THE BREEDING SEASON

By D. I. D. Howie

Zoology Department, Trinity College, Dublin

(Text-figs 1 and 2)

As a result of work by Pirlot (1933), Newell (1948), Smidt (1951) and Duncan (1953) it has become accepted that on European coasts the lugworm has a restricted breeding season occurring only in the autumn. Previously, several workers had stated that this species also spawns in the spring. Kyle (1896) reported that at St Andrews spawning took place between January and March and again between July and September. Similarly, Gamble and Ashworth (1898) and Ashworth (1904) found that on the Lancashire coast the laminarian variety spawned in the spring, although the littoral variety spawned during late summer.

Pirlot (1933), Newell (1948) and Duncan (1953) also suggest that there is some relationship between the onset of spawning and a particular phase of the tidal cycle. Thus, Pirlot (1933) observed spawning during three seasons on the Belgian coast; on each occasion there was a 2-day spawning crisis falling on either full or new moon spring tides. He suggested that the onset of spawning is influenced by the moon acting through tides. Newell (1948), in a comprehensive review of the life history, found that at Whitstable, although spawning started during springs, the peak of spawning occurred during neaps. Duncan (1953) collected data from many areas around the British Isles and, with the exception of St Andrews, again found that the peak of spawning took place during neap tides. Duncan also noted that spawning occurred later in the year on the west coast of Britain and in Ireland than on the east coast of Britain, and suggested that in any one area spawning takes place at about the same time each year.

In the present paper, the relationships between environmental conditions and the onset of the breeding season are analysed in greater detail and the possibility of a spring breeding season re-examined.

Lugworms were collected at intervals during the years 1949–53 at St Andrews, and 1953–57 (excluding 1956) at Dublin. Mr E. Latham of the Marine Laboratory, Millport, supplied worms from Fairlie Sands and Kames Bay during the spring of 1958.

The terms *laminarian* and *littoral* are used here merely to indicate whether the worms were collected at a low level (L.W.N.T.—L.W.S.T.—laminarian) or high level (H.W.N.T.–L.W.N.T.—littoral) on the shore. Both littoral and laminarian forms were obtained at St Andrews; while near Dublin, littoral forms were collected at Booterstown, and laminarian forms at Seapoint. The worms supplied from Millport were laminarian forms.

The earlier part of this work was carried out at the Gatty Marine Laboratory, St Andrews. Grateful acknowledgement is made to Dr J. M. Dodd for help and advice, and to the Carnegie Trust for a grant in aid of research. The author is also indebted to the Meteorological Office of the Air Ministry, Edinburgh, for sea temperature records from the Bell Rock Lighthouse, off St Andrews, and air temperatures from the R.A.F. station, Leuchars near St Andrews. The Meteorological Service, Department of Industry and Commerce, Dublin, kindly supplied air temperature records from Dublin Airport (Collinstown).

THE SPRING BREEDING SEASON

The opportunity was afforded at St Andrews to re-examine, in the same area, the work of Kyle (1896) who reported the spawning of *Arenicola marina* in the spring. Lugworms were collected during February, March and April in each year from 1949 to 1953. None of the worms examined contained genital products, and there was no evidence of spawning taking place.

As regards Millport, the author is indebted to Dr J. D. Robertson of the University of Glasgow and Mr E. Latham of the Millport Marine Station for the following observations (personal communications). Robertson states that he received '*Arenicola* with near-ripe eggs and sperm' from Millport on 30 March 1956 and on 19 March 1957. Latham agreed that 'around Easter spring tides *Arenicola* collected from Fairlie Sands were ripe', but he also mentioned that specimens from Kames Bay spawned later in the year. As a result of these observations, samples were obtained from Millport at approximately monthly intervals in the spring of 1958. On 10 March, fourteen specimens were obtained from Kames Bay. None of these contained genital products. In contrast, fourteen specimens collected from Fairlie Sands on the same date all contained genital products. Of these, five females contained eggs approaching maturity (average diameter 100–180μ) while three females contained 'mature' eggs (average diameter 180–190μ). Three males contained sperm cells approaching maturity (40–80% in morulae) while three were immature (0–40% in morulae). The stage in development of the sexual products of males can be estimated by counting the relative numbers of rosettes and morulae (sperm plates) in thin films of coelomic fluid. Morulae gradually replace rosettes as spawning approaches. Newell (1948) describes the stages in the development of the germ cells in the body cavity.

In the further collection of twenty-seven worms from Fairlie Sands on 9 April, twenty-one contained genital products approaching maturity or were mature. There was one immature specimen and five specimens which were thought to be spent. Spent females contained a few residual eggs, irregular in size; spent males contained a few morulae. In both sexes, there was an unusual amount of dark granular material in the body fluid.

There was a marked change in a sample obtained on 19 May (Table 1). Of twenty-five worms, eighteen were spent, only three contained genital products approaching maturity or 'mature' while four specimens were immature. This pattern was repeated in a sample obtained on 3 June, except for an increase in the number of immature specimens (Table 1).

TABLE 1. SAMPLES FROM FAIRLIE SANDS, MILLPORT, DURING SPRING OF 1958

Date	No. of worms	Immature[1]	Approaching maturity[2]	'Mature'[3]	Spent or no genital products
10. iii. 58	14	3	8	3	0
9. iv. 58	27	1	14	7	5
19. v. 58	25	4	1	2	18
3. vi. 58	30	12	2	0	16

[1] Gametocytes or eggs: 40–100 μ. Sperm: 0–40 % morulae.

[2] Eggs 100–180 μ. Sperm: 40–80 % morulae.

[3] Eggs 180–190 μ. Sperm 80–100 % morulae.

TABLE 2. DEVIATIONS IN ANNULATION AND SEGMENT NUMBER AMONG SPECIMENS OF *ARENICOLA MARINA* FROM FAIRLIE SANDS, MILLPORT

	Annulation formula[1]		Chaetigerous segments		
No. of specimens	i. 2, ii. 3, iii. 4	i. 2, ii. 2 or 2½, iii. 4	Incomplete 19	Complete 19	Incomplete 20
96	90	6	6	85	5
Percentage	93·75	6·25	6·25	88·5	5·25

[1] See Wells, 1957.

The specimens from Fairlie Sands were examined anatomically. All were large (trunk = 19 cm average length), dark worms and were collected from the laminarian zone. This suggests that they might belong to the spring-spawning 'laminarian variety' defined by Gamble & Ashworth (1898) and Ashworth (1904). However, a very large proportion of the worms from Fairlie Sands displayed the annulation of the first chaetigerous segments described for their 'littoral variety', i.e. i. 2, ii. 3, iii. 4 (formula after Wells, 1957, see also Table 2).

No evidence was thus found to support Kyle's (1896) report of a spring breeding season at St Andrews. However, there is no doubt that a large proportion of the laminarian population on Fairlie Sands, Millport, spawns in the spring (mainly between 9 April and 19 May in 1958). The immediate appearance of immature worms (containing gametocytes) after the spring breeding season at Fairlie Sands suggests that at least a proportion of this population spawns again later in the year. In view of the fact that Gamble & Ashworth (1898) and Ashworth (1904) gave accurate accounts of oviposition when describing a spring spawning among lugworms on the Lancashire coast, it is surprising that little credence has been given to their work in recent years. Although Duncan (1953) reported autumn breeding among nearby populations (Wales, Isle of Man), this does not invalidate their observations. As shown above, the Fairlie Sands population probably breeds both in spring and autumn, while the adjacent Kames Bay population breeds only in the autumn. It may well be then, that at least on the west coast of Britain, there are isolated spring-spawning populations other than that described here. In common with worms from all other areas studied in recent years (Wells, 1957; Brewster, unpublished), the vast majority of the worms from Fairlie Sands display the characters of the 'littoral variety' as defined by Gamble & Ashworth (1898). It appears, therefore, that the occurrence of a spring spawning at Millport is unrelated to varietal differences.

Southward & Southward (1958) have recently reviewed breeding in the Arenicolidae. The belief that *A. marina* spawned only in the autumn led them to suggest that this species conforms to Orton's (1920) dictum that species towards the southern limits of their distribution should breed only in the colder months of the year. Despite the account of a spring breeding season given here, this statement is still largely true. It is significant that the spring-spawning population at Millport is laminarian in situation. It is suggested that the higher temperatures experienced by these forms, due to their greater coverage by the tide, permits gamete formation to go forward during the winter. If this is true, it might be expected that spring spawning would be common among laminarian populations. However, the larval stages of *A. marina* are found in the surface sand towards the upper reaches of the shore (Newell, 1948). It is unlikely that the larvae of a cold-water species would be able to survive the high summer temperatures experienced in this habitat, particularly towards the southern limits of its distribution. This hypothesis would account for the possibility of gamete production during the winter and at the same time the rarity of spring-spawning populations in Britain. It may be that, due to local factors, larvae of *A. marina* find an unusually sheltered habitat on Fairlie Sands.

THE AUTUMN BREEDING SEASON

The percentages of worms containing genital products in the collections made at St Andrews and Dublin during late September, October and November are given in Tables 3 and 4 and shown against minimum air temperatures and tidal conditions in Figs. 1 and 2. The air temperatures given are the lowest *at low tide* in periods of 5 days. When spawning was not observed on the shore the appearance of spent worms in the collections, i.e. a decrease in the percentage of worms containing genital products, indicated that breeding was taking place.

St Andrews

The date on which spawning commenced was most accurately determined in 1951 and 1952. A few sperm puddles were observed on the shore on 29 and 31 October 1951. It was evident that spawning had just commenced as few of the worms collected on these dates were spent (Table 3). The peak of spawning occurred on 1 and 2 November and spawning continued up to 14 November, when less than 5% contained genital products (Fig. 1A). In 1952, 527 worms were collected between 7 and 14 October; there was no evidence of a drop in the percentage containing genital products (Table 3) and no sign of spawning on the shore. On the 16th to 19th inclusive, sperm puddles appeared all over the shore (the peak) and the percentage of mature worms decreased sharply. Spawning activity had very much decreased by the 20th and 21st.

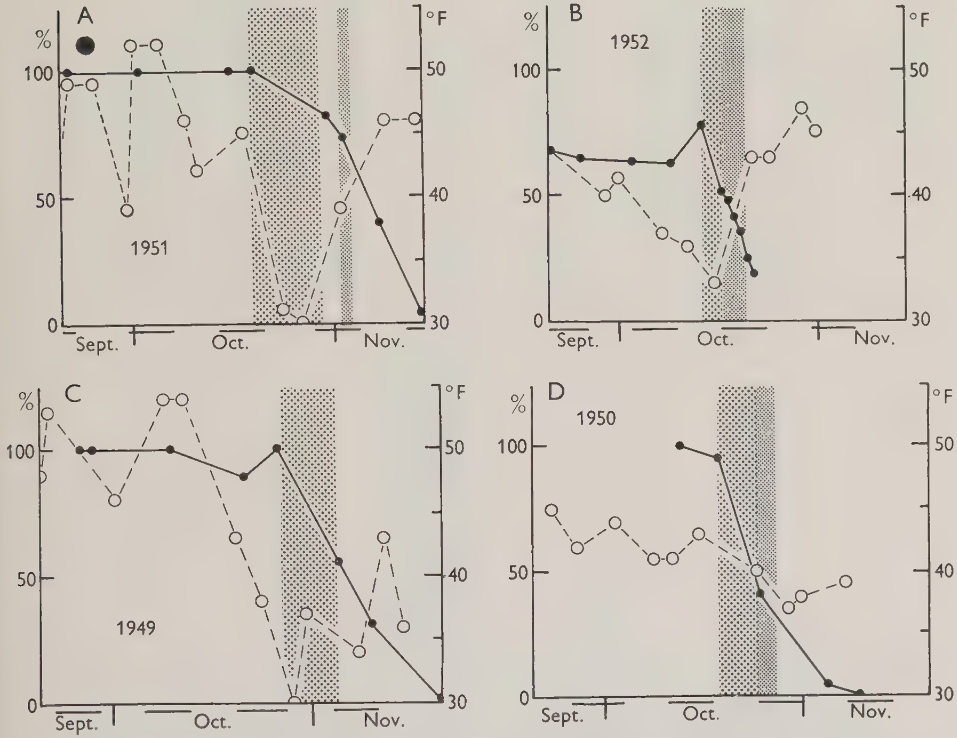


Fig. 1. The breeding season of *Arenicola marina* at St Andrews related to minimum air temperatures and tidal conditions. ○- - -○, minimum air temperatures (° F); ●——●, percentage of worms containing genital products; Large stippling, period during which spawning commenced; fine stippling, time at which peak of spawning was observed on the shore. A double line on the abscissa indicates a spring tide period.

The breeding seasons in these years were similar in that (*a*) they commenced towards the end of a period of neap tides, (*b*) the peak was mainly during the succeeding full moon springs, and (*c*) the beginning of spawning coincided with a sharp fall in minimum air temperatures; this being the first drop to the seasonal minimum in each year (Fig. 1 A, B).

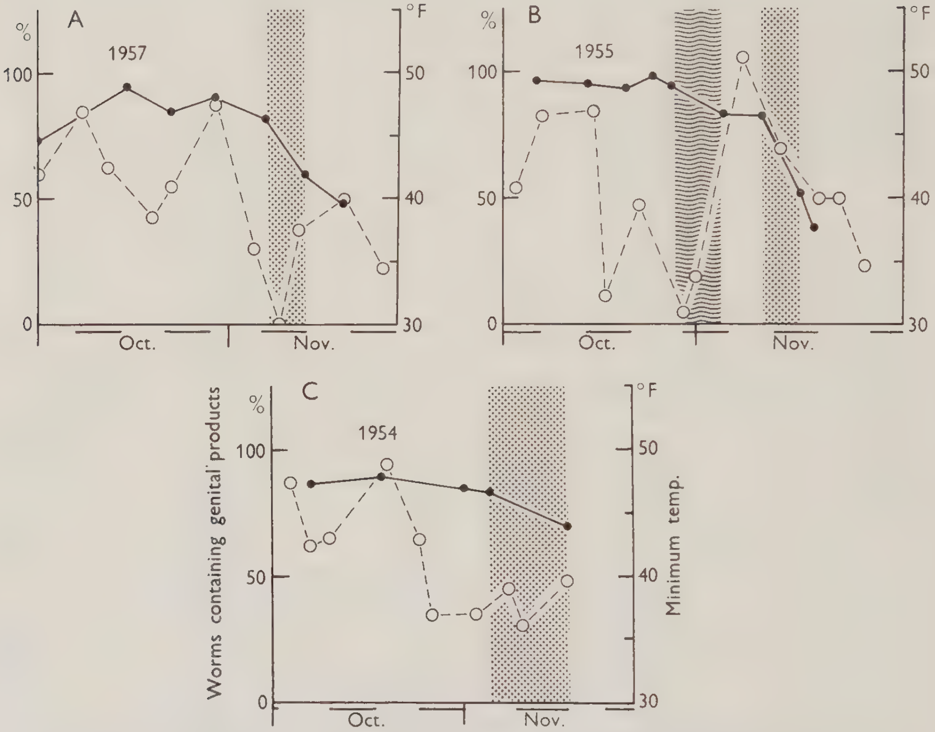


Fig. 2. The breeding season of *Arenicola marina* at Dublin (Booterstown). (Wavy lines indicate period of an early slight spawning; otherwise as for Fig. 1).

The seasons differed in relation to weather conditions. In 1951, spawning began in calm weather but storms developed during the peak. In 1952, the weather was calm throughout.

In 1949, collections were smaller and observations were made less frequently. Spawning was not observed, but collection data show that it commenced between 24 October and 3 November, i.e. probably during neap tides (Fig. 1 C). Spawning again coincided with a sharp fall in minimum temperatures. In 1950, collections were made principally from a laminarian population, and it was this population which was observed spawning on the shore. Spawning appears to have commenced between 18 and 24 October (neaps) and the peak was observed on the 24th to 26th inclusive (springs). These data support the tidal relationships observed in 1949, 1951 and 1952. While there was a gradual fall in minimum air temperatures prior to spawning (Fig. 1 D) there was no sharp decrease similar to that observed in the other years mentioned. The spawning of laminarian forms will be discussed as a special case below.

In 1949 the breeding season was characterized by intermittent storms, while in the following year it was calm at first but stormy later in the season.

TABLE 3. RECORDS OF COLLECTIONS AT ST ANDREWS

The 1949–51 records are compounded from two populations which differ in the percentage of the total population normally containing genital products in the summer. This percentage is therefore in each case treated as 100 % and any fall in the number of worms containing genital products is percentaged accordingly. The 1952 figures are from a single population and actual percentages are given.

	Date	Total worms examined	Percentage containing genital products	Spawning observed
1949	26–28. ix.	54	100	—
	9. x.	9	100	—
	20, 21. x.	75	89	—
	24–27. x.	31	100	—
	3–5. xi.	85	56	—
	9. xi.	58	31	—
	20. xi.	20	0	—
1950	12. x.	40	100	—
	18. x.	36	94	—
	23–26. x.	52	41	+ (peak)
	4. xi.	64	5	—
	9. xi.	9	0	—
1951	20. ix.	44	100	—
	1. x.	25	100	—
	14–16. x.	25	100	—
	18. x.	21	100	—
	29–31. x.	89	82	+
	1, 2. xi.	91	73	+ (peak)
	7. xi.	34	41	+
	13–14. xi.	109	4	+
1952	19, 20. ix.	223	68	—
	23–25. ix.	328	65	—
	1–3. x.	441	64	—
	7–9. x.	320	63	—
	12–14. x.	207	78	—
	16. x.	201	52	+ (peak)
	17. x.	311	48	+ (peak)
	18. x.	164	42	+ (peak)
	19. x.	75	36	+ (peak)
	20. x.	141	25	+
	21. x.	106	18	+

Dublin (Booterstown)

Spawning in this littoral population was never observed on the shore, but the beginning of the breeding season in 1955 and 1957 can be determined within narrow limits from collection records. Taking 1957 first, the first significant drop in the percentage of worms containing genital products occurred between 6 and 12 November (Table 4). Thus, unlike the above observations at St Andrews, spawning commenced during full moon springs (Fig. 2A). However, the beginning of spawning was again marked by a fall in minimum temperatures.

The position was more complicated in 1955. In regular collections during the summer (of approximately 60 worms/collection) the percentage of the total worm population containing genital products varied between 93% and 98%. Between 27 October and 4 November this percentage dropped to 83%.

TABLE 4. RECORDS OF COLLECTIONS FROM A SINGLE POPULATION AT BOOTERSTOWN, DUBLIN

	Date	Total worms examined	Percentage containing genital products
1957	30. ix.	41	73
	15. x.	38	95
	22. x.	49	85
	29. x.	43	91
	6. xi.	40	82
	12. xi.	50	60
	18. xi.	38	48
1955	6. x.	32	97
	14. x.	57	95
	20. x.	59	93
	24. x.	50	98
	27. x.	70	94
	4. xi.	44	83
	9, 10, 11. xi.	125	82
	16. xi.	60	52
	18. xi.	72	38
1954	6, 8. x.	45	87
	18. x.	31	90
	31. x.	72	85
	4. xi.	36	83
	15. xi.	45	69

In view of the lack of variation in sampling during the season, this drop in the percentage of worms containing genital products was interpreted as indicating the beginning of spawning. There was, however, no further increase in the number of spent worms up to 11 November (Table 4). The main spawning appears to have commenced between 11 and 16 November, during which time the percentage of worms containing genital products fell rapidly to 52%. Both the slight earlier spawning and the main spawning appear to have commenced during spring tides (Fig. 2B). Minimum air temperatures fell abruptly to 32° F on 16–18 October and there ensued a period of cold weather with the lowest temperature (31° F) occurring on 29 October. This coincided with the first slight drop in the percentage of worms containing genital products. Thereafter, air temperatures rose rapidly; the lowest recorded (at low tide) during the next 10 days was 51° F. This was followed by a second period of falling temperatures which coincided with the beginning of the main breeding season (Fig. 2B).

Only a limited amount of data is available for the 1954 season (Table 4). The first major drop in the percentage of worms containing genital products occurred between 4 and 15 November, but this drop is of doubtful significance as no further collections were made. This period was preceded by a sharp fall in minimum air temperatures, but in this case only to 37° C. There was no record of spawning at Booterstown in 1953 (see below).

Dublin (*Seapoint*)

The breeding season of the laminarian population at Seapoint was never fixed with accuracy, but the following observations were made during 1953, 1954 and 1957. In 1953, 75% of the Seapoint population were spent on 4 November, although there was no indication of the Booterstown population even beginning to breed as late as 12 November, when collecting was discontinued. On 9 November 1954, 89% of the Seapoint worms were spent when breeding was just beginning at Booterstown. Finally, in 1957 there was evidence to show that breeding was under way around 9 October at Seapoint, and in this case the season did not commence at Booterstown until 6/12 November. Although in each year, spawning at Seapoint was preceded by gradually falling temperatures (as at St Andrews in 1950) the minimum recorded prior to spawning (37°–40° F) was not so low as that usually associated with spawning in littoral populations.

DISCUSSION

The evidence of Newell (1948) and Duncan (1953) that in most areas around the British Isles autumn-spawning *Arenicola* begin to breed during spring tides, while the peak of spawning occurs during neaps, cannot be disputed. Although spawning was not observed on the shore at Dublin, and in consequence little can be said about the occurrence of the peak, evidence that spawning commences during spring tides tends to support their observations. On the other hand, there is no doubt that in some areas, e.g. St Andrews, the opposite occurs. Spawning begins during neaps, and its peak occurs during springs. If Pirlot's (1933) 'spawning crisis' is equivalent to the peak of spawning his evidence supports this statement. This variation suggests that tidal or lunar conditions cannot alone control the onset of spawning in *Arenicola*. Similarly, rough and calm weather vary in relation to the breeding season. Observations made at St Andrews again agree with Pirlot in this respect.

The present paper was stimulated by the subjective observation, over many years, that spawning at St Andrews is first seen on frosty mornings. Examination of the meteorological records presented in this paper, shows that there is an apparent correlation between the first fall in air temperatures to the autumnal minimum and the onset of spawning, at least in littoral populations.

Whether this correlation has any significance depends in part on the extent to which *Arenicola* may be exposed to a sudden fall in air temperatures during the intertidal period. Southward (1958) has shown that, in winter, during daylight, air temperatures close to rocks on the shore and the body temperatures of animals are generally higher than screen air temperatures read even at a nearby site. However, on an isolated occasion when there appears to have been a sudden cold spell (18 November 1953, Table 4, p. 59) Southward shows that at 10 a.m., after 7 h exposure, screen air temperatures, shore temperatures and the average body temperatures of barnacles and top shells lay within the range 6·8° C to 7·9° C. Previously, Bruce (1928) had stated that at 9 a.m. G.M.T. air temperatures and the surface temperatures of beach sands are approximately isothermal. With a low tide at night or in the early morning, there may, therefore, be little mitigation of a sudden and severe fall in air temperature on the sand. Further, during cold weather in the autumn, the sand surface must be subjected to repeated changes in temperature between tidal and intertidal periods as even inshore waters are still relatively warm (Bruce, 1928; Southward, 1958). It might still be argued that by their burrowing habit lugworms escape these changes of surface temperature. Bruce (1928) has shown that at 20 cm the temperature of the sand is similar to that of inshore water. However, Wells (1945, 1949) has suggested that during the intertidal phase the lugworm makes periodic excursions towards the surface in the course of 'aerial respiration' or to test the temperature and salinity of the surface water. There seems little doubt that in this way the lugworm is exposed to surface conditions.

It is suggested, therefore, that the spawning of autumn-breeding *Arenicola* is stimulated, at least indirectly, by the first fall in air temperature to the seasonal minimum. The effect of this fall in temperature is emphasized by the marked difference between sea and air temperatures at that time. In view of the occurrence of a spring breeding season, reported in this paper, it may later be proved that it is merely a distinct change in temperature which is required to initiate spawning once the worms have matured. This problem is now being investigated experimentally.

Contributory evidence for the above hypothesis may be found in the fact that as the eggs develop in the coelom, they rapidly assume the size of mature eggs but are retained in the body cavity for some weeks before spawning actually takes place. A similar observation has been made by Newell (personal communication). This seems to support the idea that spawning is induced by a sudden stimulus such as temperature change rather than by rhythmic tidal influences.

The relatively early spawning of laminarian forms in response to less marked falls in temperature could be explained by the fact that these worms, covered for prolonged periods during neaps, are conditioned to relatively constant temperatures. Thus when exposed during spring tides they may respond to a fall in temperature much less violent than that required to initiate spawning in their littoral neighbours. In this case, there would be an additional element of tidal control.

Although spawning will continue in the face of rising temperatures (Figs. 1 and 2), there may be a fixed temperature above which spawning will not take place. Thus in 1955 (Fig. 2B) slight spawning appears to have coincided with a fall in temperature and then ceased for a period of 7 days, during which the weather was unusually warm (minimum air temperature 51° F). Subsequently, there was a second fall in temperature and the main breeding season commenced. It has also been found that experiments on the artificial stimulation of spawning in *Arenicola* fail at about 57° F (14° C) but the results improve below 53° F (12° C) (unpublished work).

The above hypothesis for the environmental control of spawning in *Arenicola* might explain some of the observations in Duncan's (1953) paper. The earlier spawning among populations on the east coast of Britain than on the west coast may be due to the earlier occurrence of low temperatures on the east coast. The onset of spawning in any one area at about the same time each year may be due to the fact that the first major drop in temperature during the autumn in any given place tends to occur during the same fortnight to three weeks every year (Figs. 1 and 2).

SUMMARY

A spring spawning occurs among *Arenicola marina* on Fairlie Sands, Millport. Although laminarian in situation the vast majority of these worms belong to the 'littoral variety'. This unusual breeding season is, therefore, unrelated to varietal differences. A hypothesis is given for the possibility and at the same time the rarity of spring-spawning populations in Britain. At St Andrews the breeding season occurs during the second fortnight of October, but is unusual in that it begins during neap tides with the peak during the following springs. Breeding in the littoral population at Booterstown (Dublin) is more conventional in that it begins during spring tides; in this case during the first fortnight in November. It is shown that over several years the breeding season of littoral populations at St Andrews and Dublin is preceded by, or coincides with, the first sharp fall in air temperatures to approximately the autumnal minimum. It is suggested that this fall in temperature provides the spawning stimulus for autumn-breeding lugworms. In view of the spring breeding season reported here, it is possibly only a distinct change of temperature which is required to initiate spawning. The earlier spawning of laminarian forms in response to a lesser temperature stimulus, may be due to an element of tidal control.

REFERENCES

ASHWORTH, J. H., 1904. *Arenicola. Mem. Lpool mar. biol. Comm.*, No. 11, 188 pp., 8 pl.

BRUCE, J. R., 1928. Physical factors on the sandy beach. Pt. I. Tidal, climatic and edaphic. *J. mar. biol. Ass. U.K.*, Vol. 15, pp. 535–52.

DUNCAN, NAN, 1953. Spawning in *Arenicola marina* (L.). *Brit. J. Anim. Behav.*, Vol. 1, pp. 85–86.

GAMBLE, F. W. & ASHWORTH, J. H., 1898. The habits and structure of *Arenicola marina. Quart. J. micr. Sci.*, Vol. 41, pp. 1–42.

KYLE, H. M., 1896. Papers from the Gatty Marine Laboratory, St Andrews. On the nephridia, reproductive organs and post larval stages of *Arenicola marina. Ann. Mag. nat. Hist.*, Ser. 6, Vol. 18, pp. 295–300.

NEWELL, G. E., 1948. A contribution to our knowledge of the life history of *Arenicola marina. J. mar. biol. Ass. U.K.*, Vol. 27, pp. 554–80.

ORTON, J. H., 1920. Sea temperature, breeding and distribution in marine animals. *J. mar. biol. Ass. U.K.*, Vol. 12, pp. 339–66.

PIRLOT, J. M., 1933. La ponte de l'*Arenicola marina*, Linné à la côte belge. *Bull. Mus. Hist. nat. Belg.*, T. 9 (No. 49), pp. 1–7.

SMIDT, E. L., 1951. Animal production in the Danish Waddensea. *Medd. Komm. Havundersøg., Kbh., Fiskeri*, Bd. 11, pp. 1–151.

SOUTHWARD, A. J., 1958. Note on the temperature tolerances of some intertidal animals in relation to environmental temperatures and geographical distribution. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 49–66.

SOUTHWARD, EVE C. & SOUTHWARD, A. J., 1958. The breeding of *Arenicola ecaudata* Johnston and *A. branchialis* Aud. & Edw. at Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 267–286.

WELLS, G. P., 1945. The mode of life of *Arenicola marina* L. *J. mar. biol. Ass. U.K.*, Vol. 26, pp. 170–207.

—— 1949. Respiratory movements of *Arenicola marina* L.: Intermittent irrigation of the tube, and intermittent aerial respiration. *J. mar. biol. Ass. U.K.*, Vol. 28, pp. 447–64.

—— 1957. Variation in *Arenicola marina* (L.) and the status of *Arenicola glacialis* Murdoch (Polychaeta). *Proc. zool. Soc. Lond.*, Vol. 129, pp. 397–419.

J. mar. biol. Ass. U.K. (1959) 38, 407–415
Printed in Great Britain

VARIATIONS IN THE ACTIVITY OF THE THYROID GLAND OF THE COD, *GADUS CALLARIAS* L., IN RELATION TO ITS MIGRATIONS IN THE BARENTS SEA

I. SEASONAL CHANGES

By A. D. Woodhead
Fisheries Laboratory, Lowestoft

(Text-figs 1 and 2)

Relatively little is known of the exact role of the thyroid gland in teleosts. Although the results of a few studies have suggested that the thyroid influences respiratory metabolism, as in the higher vertebrates, a greater number of experiments have failed to show that either treatment of fish with thyroid hormones or inactivation of the gland produces any significant effect upon the oxygen consumption of the fish. Thyroid activity has been linked with reproduction in some fish, although there is little evidence to show that the relationship is more than coincidental. The thyroid has also been connected with osmoregulation, but reviewing this work Pickford (1957) concludes that the relation with osmoregulation is only a secondary or collateral one. Recently Hoar (1953, 1955) has suggested that the thyroid may control the migratory response of fish. However, in the migratory species which have been studied in detail, the salmon, *Salmo salar* L. and *Onchorhynchus* spp. and the eel, *Anguilla vulgaris*, changes in the salinity of the environment during their migrations were complicating factors since salinity changes alone have been shown to stimulate thyroid activity in non-migratory fish (Olivereau, 1954).

The present paper describes the seasonal cycle of activity in the thyroid gland of the cod, *Gadus callarias* L., in the Barents Sea, and is one of a series on the physiology and behaviour of the fish. This species is of particular interest because of the great length of its monadromous spawning migration, the adults often travelling more than 800 miles before reaching the spawning grounds. The distribution and movements during the course of the life cycle have been described in detail (Trout, 1957).

The cod mature for the first time at an average age of 8 or 9 years old. Throughout the summer months, they are found on the shallow-water feeding grounds of the Bear Island–Spitsbergen Banks. In late September, at the onset of maturation, the ripening fish begin to move south and west to leave the shelf, and migrate to the spawning grounds inside the Lofoten Islands on the north Norwegian coast. Spawning occurs each year from mid-February until mid-April. After first maturity, the cod spawns annually, the spent fish dispersing to the feeding grounds where they remain until the following autumn. The young larvae are distributed from the spawning grounds over large areas of the Barents Sea by surface currents. Lundbeck (1932) suggested that they become migratory at 3 years old. From length measurements of immature cod caught by the commercial trawlers, Maslov (1944) demonstrated a pattern of migration which has since been confirmed and extended (Trout, 1957). During the summer months, June to September, the immature fish are found in large numbers in the shallow-water feeding grounds of the Bear Island–Spitsbergen Bank. In autumn the fish begin to leave these grounds and migrate into deeper waters around the shelf where they remain throughout the winter, until the late spring, when they return to the Banks. This migration is repeated annually until the fish mature.

MATERIAL AND METHODS

The thyroid glands were collected from the cod, *Gadus callarias* L., in the Barents Sea. Mature and immature fish, from 50 to 120 cm. were selected from the catch of the M.A.F.F. Research Vessel 'Ernest Holt'. All the fish used in this investigation were caught at temperatures of between 1° and 6° C and at salinities of 34·5 to 35·2‰. The cod were killed by a sharp blow on the head; a sample of blood was taken for chemical analysis, and the thyroid was then removed. In the cod, as in the majority of teleosts, the thyroid is not encapsulated, but consists of a number of discrete follicles, closely apposed to the ventral aorta, and lying in the surrounding connective tissue. The aorta was therefore dissected out, from the heart to the level of the first gill arch; this ensured that most of the gland was removed. The thyroids were fixed in Bouin's fluid for 24 h, routinely dehydrated and embedded in paraffin wax. Serial sections were cut at 4μ, and alternate slides stained with haemotoxylin and eosin, and Heidenhain's azan.

TABLE 1. FISHING DATES, 1956–7

8–26 August
8–20 September
7–23 October
20 November–5 December
20–31 January
10–26 March (Spawning sample)
26–31 March (Norway coast)
23–30 April
1–10 June
16–31 July

A quantitative value for the activity of the gland was obtained from measurements of the follicular cell height. Pickford & Atz (1957) have reaffirmed the reliability of this method of estimating the activity of the thyroid. In order to ensure that the same follicle was not measured twice, every twentieth section was selected for study. Four diametrically opposite cells were measured in each follicle and not less than two hundred readings were made on each fish.

Samples were obtained throughout the year, at times corresponding to the major phases of the migratory and reproductive cycles of the fish. The dates of capture are given in Table 1. Each sample consisted of ten fish, five males and five females. A statistical analysis of the results showed that there was no difference in the activity of the gland between the two sexes at any time of the year. The state of maturity was determined by examination of the gonads as the fish were dissected; in addition, the gonads were fixed, and sections studied under the microscope.

The results presented below, and in the following paper, are based on between 40,000 and 50,000 follicular cell height measurements.

RESULTS

Adult cod

The mean values for the cell height measurements of the thyroids of the mature cod are given in Fig. 1 and Table 2. The reaction of the colloid to azan staining confirmed the results obtained from cell height measurements. In resting glands the colloid stained a deep red, and had a dense laminated appearance. The surrounding follicular cells were low and stretched round the stored colloid: frequently the nucleus caused a distinct bulge in the centre of the cell. The cytoplasm of the follicular cells stained a uniform pink.

TABLE 2. SEASONAL VARIATIONS IN THE MEAN FOLLICULAR CELL HEIGHT IN THE THYROID OF THE ADULT COD

Sample	No. of fish	Mean follicular cell height (E.P.U.)	Standard deviation
August	10	47·8	5·1
September	10	61·5	6·5
October	10	69·0	6·1
November–December	10	76·9	4·3
January	10	81·5	7·5
March (Spawning)	10	70·0	3·6
March (Coast)	10	67·7	5·5
April	10	54·4	3·4
June	10	50·1	2·4
July	10	54·1	4·6

75 E.P.U. = 10 μ

In active glands the colloid stained blue and appeared granular. The follicular cells were high and columnar, with a large round nucleus situated at the distal end of the cell. Cytoplasmic granules, staining a deep pink, were often present, usually surrounding the nucleus, and extending half way down the cell. The inner edge of the cell near the follicle stained much more faintly. Deep red droplets were sometimes seen at the outer edge of the colloid. In some active thyroids the follicular membrane had ruptured, and the colloid was discharged out of the follicle. Other follicles had been invaded by leucocytes.

Throughout the spring and summer months, from April to late September, the follicular cells were low, and the thyroid appeared, histologically, to be in an inactive state. However, during this period, there was a small but significant ($p = 0{\cdot}03$) increase in follicular cell height. Marked secretory activity began in late September. The height of the follicular cells continued to increase throughout the winter, reaching a maximum in January, prior to spawning. The follicular cell height was less in fish caught in March at spawning time. The thyroid glands of cod caught some 50 miles north of the spawning grounds, and in which the gonads were ripe but not yet running freely with spawn, showed no significant difference in cell height from thyroids of 'running-ripe' cod caught on the spawning grounds, inside the Lofoten Islands. In spent fish the gland re-entered the resting condition.

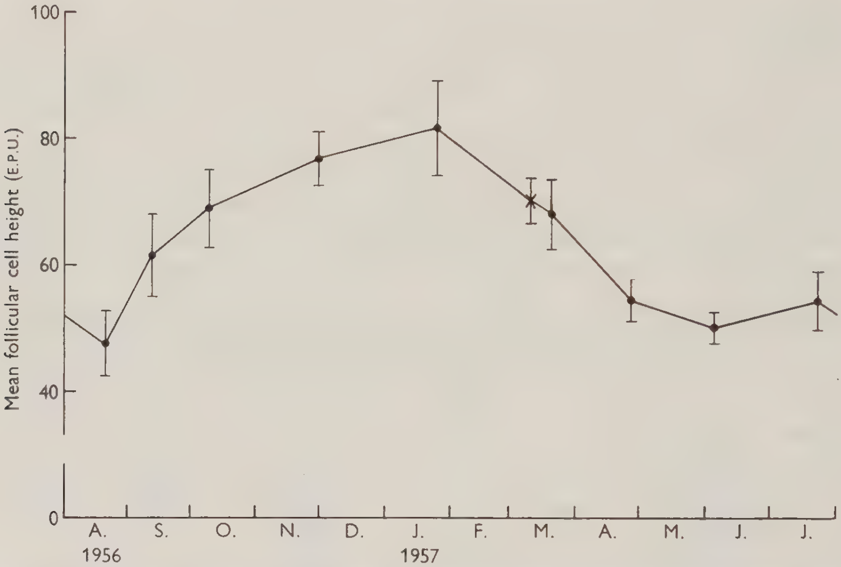


Fig. 1. The seasonal variation in the mean follicular cell height of the thyroid gland in adult cod. ×, ripe cod caught on the spawning grounds inside the Lofoten Islands. The follicular cell height is expressed in eyepiece units (E.P.U.).

A histological study of the gonads showed that throughout the summer these organs were quiescent. Maturation began in late September with the enlargement of the eggs, and the production of primary spermatocytes; at the same time the thyroid became active. Maturation continued throughout the winter migration, the gonads being fully ripe by March, as the fish reached the spawning grounds on the North Norwegian coast.

Immature cod

The cycle of activity in the thyroid of the immature cod is shown in Fig. 2 and Table 3.

The gland became active in September, as in the adult fish, but the maximum cell height was reached in December, after which the follicular cell height decreased, returning to the resting state by April. A second smaller peak of activity occurred in July, as in the adult fish. The magnitude of the thyroid cycle of the immature fish was of a lesser order than that of the adults.

The gonads of the immature fish showed no changes throughout the year. The ovaries and testes remained small, and were filled with primary germ cells.

TABLE 3. SEASONAL VARIATIONS IN THE MEAN FOLLICULAR CELL HEIGHT IN THE THYROID OF THE IMMATURE COD

Sample	No. of fish	Mean follicular cell height (E.P.U.)	Standard deviation
August	10	48·4	4·5
September	10	58·3	6·2
October	10	66·5	6·5
November–December	10	67·9	7·2
January	10	64·8	8·7
March	10	55·1	3·2
April	10	49·5	4·5
June	10	48·4	4·5
July	10	53·6	4·3

75 E.P.U. = 10 μ

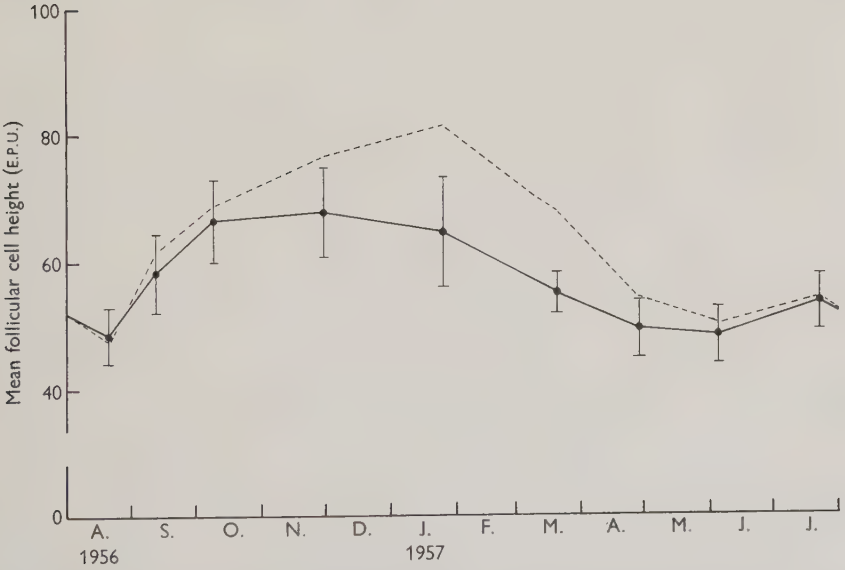


Fig. 2. The seasonal variation in the mean follicular cell height of the thyroid gland in immature cod. The broken line gives the seasonal variation in adult cod for comparison. The follicular cell height is expressed in eyepiece units (E.P.U.).

DISCUSSION

Barrington & Matty (1954) have suggested that there is a correlation between the activity of the thyroid gland and the phases of the reproductive cycle in fish. They found that in the minnow, *Phoxinus phoxinus* L., the thyroid was maximally active at spawning time, the activity decreasing as spawning ceased. Similarly, in the pike, *Esox lucius* (see Zaitzev, 1955), and in the herring, *Clupea harengus* (see Buchmann, 1940), there is increased thyroidal activity at spawning time. Lieber (1936) associated a period of thyroid activity prior to spawning, with the enlargement of the gonads in *Misgurnus fossilis*; a second period of thyroid activity occurred after spawning, but the gland was less active during the actual spawning period.

Laboratory experiments in which fish have been maintained in antithyroid drugs have suggested that these substances may have an inhibiting effect on maturation of the gonads. Chambers (1951) found that the testes of *Fundulus heteroclitus* kept in a solution of thiourea had regressed. Barrington & Matty (1952) working with the minnow, and Smith, Sladek & Kellner (1953) with *Lebistes reticulatus* achieved similar results. However, in a further series of studies on the minnow, Barrington (1954) reported that he could stimulate the development of the testes in winter, by artificial light, although the fish were immersed in thiourea, and Hoar (1939) in his studies on the Atlantic salmon, *Salmo salar*, found no evidence of an increase in thyroid activity which could be related to spawning in the ripe male parr.

Treatment of fish with thyroxine has given contradictory results. Thus Hopper (1952) and Gaiser (1952) were able to induce the early development of the secondary sexual characters in *Lebistes reticulatus* whilst Smith, *et al.* (1953) obtained no response with the same species. Kalashnikov & Skadovskii (1940, 1948), studying the degeneration of ova in prespawning female Acipenserines kept in captivity, found that administration of thyroid hormone improved the condition of the gonads.

From her studies on gonadal function and thyroid activity in mammals, Brown-Grant (1956) concluded that there was little evidence to support the concept of a marked association between the two glands. Reviewing similar results in fish, Pickford (1957) suggested that, although the presence of an active functional thyroid might be a requisite for the maturation of the gonads, only a low level of thyroidal activity may be necessary. The present observations do not negate the suggestion that there is a relationship between the thyroid gland and the gonads. In the adult cod, the two events occur simultaneously, gonadal maturation beginning as the thyroid becomes active, and the thyroid returning to a resting condition in the spent fish. But these results do not indicate whether the activity of the thyroid is necessary for gonad maturation, or whether the occurrence of the two events is coincidental. However, a similar cycle of activity occurred in the thyroid of the immature cod, although the gonads remained quite inactive throughout the cycle.

The results of the present work suggest that the thyroid cycle in the cod may be related to the migratory activity (see Woodhead, 1959, p. 421 of this *Journal*). In both adult and immature fish, the cycle of activity in the thyroid began at the start of their southward migration over the Bear Island–Spitsbergen Bank, and declined as the migration was completed. The thyroid remained active in the adults until March, when the cod reached the Lofoten Island spawning grounds, but in the immature cod, the activity had already begun to fall by January, when most of the fish were completing their migration to the overwintering grounds.

The spawning migration of the adult cod and the overwintering migration of the immature fish are active contranatant migrations against the West Spitsbergen current. In contrast, it has been suggested that the northward return of the spent cod to the feeding grounds might be accounted for by the passive carriage of the fish within the water mass (Trout, 1957). Perhaps the simplest explanation of the active contranatant migration would be that there was a change in the reaction of the fish to water currents, probably accompanied by an increase in the general level of locomotory activity. Since fish readily orientate against a strong water current, if provided with adequate sensory clues, it seems likely that the change in the reaction of the cod to current would be in the nature of a lowered threshold for initiation of the response, rather than a completely new response. The passive denatant return of the spent fish to the feeding grounds could similarly be explained by a rise in the threshold for the reaction to current, and probably a fall in the general level of activity, the fish swimming randomly within the northward moving waters of the Norwegian current. Trout (1957) states that the cod shoals are largely pelagic at this time, which would facilitate this passive displacement.

Fontaine (1954) has emphasized the role of internal factors in the causation of migration in fish and Hoar (1955) has suggested that the secretory activity of the thyroid gland and gonads may be responsible for increased locomotory activity, sometimes involving long migrations, associated with reproduction. Hoar *et al.* (Hoar, Mackinnon & Redlich, 1952; Hoar, Keenleyside & Goodall, 1955) have shown an increased level of locomotor activity in fish maintained in thyroxine solutions, and decreased activity in thiourea; immersion in solutions of sex steroid hormones also increased the general level of activity. In the minnow, *Phoxinus phoxinus*, Woodhead (1956) has shown that during mid-April, prior to spawning, the mean swimming speed was 159% higher than in December; in April the thyroid would be maximally active (Barrington & Matty, 1954), the gonads would also be secreting hormones since the minnows had begun to assume nuptial coloration, and both gonadal and thyroid hormones may have contributed to the increased swimming speed observed in the minnow.

In the adult cod, the gonads were ripening throughout the spawning migration, when the thyroid gland was active, and the action of the thyroid and gonadal hormones may have been additive, both causing an increase in migratory activity. However, the results obtained with the immature cod, in which the gonads remained inactive, suggest that an increase in the activity of the thyroid gland alone may initiate and sustain a lengthy migration.

I would like to thank my husband for collecting the material used in this study, and for his encouragement whilst the work was in progress. Dr F. R. Harden Jones very kindly collected the sample from the cod on the Lofoten spawning grounds. Gratitude is also due to Mr Michael Graham, C.M.G., and to Mr R. S. Wimpenny, O.B.E., for their continued support of my work.

SUMMARY

A seasonal cycle of activity has been demonstrated in the thyroid gland of both adult and immature cod. The gland became active in late September and activity continued in the adults until spawning time in March; in spent cod the gland had re-entered the resting condition. In the immature fish activity had declined by March.

In the adult cod the thyroid gland was active throughout the period of maturation of the gonads, but the present results did not indicate whether these events were causally related or coincidental; a thyroid cycle occurred in the immature cod, although the gonads remained quiescent.

It has been suggested that thyroid activity may initiate and sustain the active migration of the cod.

REFERENCES

Barrington, E. J. W., 1954. Hormones and control of zoological function. *Nature, Lond.*, Vol. 174, pp. 720–22.

Barrington, E. J. W. & Matty, A. J., 1952. Influence of thiourea on reproduction in the minnow. *Nature, Lond.*, Vol. 170, p. 105.

—— —— 1954. Seasonal variation in the thyroid gland of the minnow, *Phoxinus phoxinus* L., with some observations on the effect of temperature. *Proc. zool. Soc. Lond.*, Vol. 124, pp. 89–95.

Brown-Grant, K., 1956. Gonadal function and thyroid activity. *J. Physiol.* Vol. 131, pp. 70–84.

Buchmann, H., 1940. Hypophyse und Thyreoidea im Individualzyklus des Herings. *Zool. Jb.*, Abt. 2, Vol. 66, pp. 191–262.

Chambers, H. A., 1951. Effect of thiourea on male *Fundulus heteroclitus* (Linn.). *Anat. Rec.*, Vol. 109, p. 366.

Fontaine, M., 1954. Du déterminisme physiologique des migrations. *Biol. Rev.*, Vol. 29, pp. 390–418.

Gaiser, M. L., 1952. Effets produits par l'administration prolongée de thioureé et de thyroxine chez *Lebistes reticulatus*. *C.R. Soc. Biol., Paris*, T. 146, pp. 496–98.

HOAR, W. S., 1939. The thyroid gland of the Atlantic salmon. *J. Morph.*, Vol. 65, pp. 257–92.

—— 1953. Control and timing of fish migration. *Biol. Rev.*, Vol. 28, pp. 437–52.

—— 1955. Reproduction in teleost fishes. *Mem. Soc. Endocrin.*, No. 4, pp. 5–22.

HOAR, W. S., KEENLEYSIDE, M. H. A. & GOODALL, R. G., 1955. The effects of thyroxine and gonadal steroids on the activity of salmon and goldfish. *Canad. J. Zool.*, Vol. 33, pp. 428–39.

HOAR, W. S., MACKINNON, D. & REDLICH, A., 1952. Effects of some hormones on the behaviour of salmon fry. *Canad. J. Zool.*, Vol. 30, pp. 273–86.

HOPPER, A. F., 1952. Growth and maturation response of *Lebistes reticulatus* to treatment with mammalian thyroid powder and thiouracil. *J. exp. Zool.*, Vol. 119, pp. 205–17.

KALASHNIKOV, G. N. & SKADOVSKII, S. N., 1940. Observations on the physiology of acipenserine fish during the period of reproduction in connection with the problem of artificial pisciculture. *Zool. Zh.*, Vol. 19, pp. 671–79 (in Russian).

—— —— 1948. Ecological and physiological study of *Sevruga* during the period of reproduction under natural and experimental conditions. *Zool. Zh.*, Vol. 27, pp. 513–24 (in Russian).

LIEBER, A., 1936. Jahrescyklus der Schilddrüse von *Misgurnus fossilis*. *Z. wiss. Zool.*, Vol. 148, pp. 364–400.

LUNDBECK, J., 1932. Biologisch-statistische üntersuchungen über die deutsche Barentsmeer—Fischerei. *Wiss. Meeresuntersuch.*, Abt. Helgoland, Bd. 18, No. 8, pp. 1–56.

MASLOV, N. A., 1944. The bottom fishes of the Barents Sea and their fisheries. *Trans. Knipovich-polyar, sci. Inst.*, Vol. 8, pp. 3–186 (in Russian).

OLIVEREAU, M., 1954. Hypophyse et gland thyroide chez les poissons. Étude histophysiologique de quelques correlations endocriniennes en particulier chez *Salmo salar* L. *Ann. Inst. océanogr. Monaco*, Vol. 29, pp. 97–296.

PICKFORD, G. E., 1957. The thyroid and thyrotrophin. In PICKFORD, G. E. & ATZ, J. W., 1957. *The Physiology of the Pituitary Gland of Fishes*. New York, Zool. Soc.

SMITH, D. C., SLADEK, S. A. & KELLNER, A. W., 1953. The effect of mammalian thyroid extract on the growth rate and sexual differentiation in the fish, *Lebistes reticulatus*, treated with thiourea. *Physiol. Zool.* Vol. 26, pp. 117–24.

TROUT, G. C., 1957. The Bear Island cod: migrations and movements. *Fish. Invest., Lond.*, Ser. 2, Vol. 21, No. 6, 51 pp.

WOODHEAD, A. D., 1959. Variations in the activity of the thyroid gland of the cod, *Gadus callarias* L., in relation to its migrations in the Barents Sea. II. The 'dummy run' of the immature fish. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 417–22.

WOODHEAD, P. M. J., 1956. The behaviour of minnows (*Phoxinus phoxinus* L.) in a light gradient. *J. exp. Biol.*, Vol. 33, pp. 258–70.

ZAITZEV, A. V., 1955. A histological investigation of the annual changes of the thyroid gland of the pike, and the neurosecretory activity of the hypothalamic nuclei in the seasonal change of the thyrotropic function of the hypophysis. *C.R. Acad. Sci. U.R.S.S.*, Vol. 104, pp. 315–8 (in Russian).

J. mar. biol. Ass. U.K. (1959) 38, 417–422
Printed in Great Britain

VARIATIONS IN THE ACTIVITY OF THE THYROID GLAND OF THE COD, *GADUS CALLARIAS* L., IN RELATION TO ITS MIGRATIONS IN THE BARENTS SEA

II. THE 'DUMMY OF RUN' OF THE IMMATURE FISH

By A. D. Woodhead
Fisheries Laboratory, Lowestoft

(Text-figs. 1–3)

Describing the migrations and movements of the cod, *Gadus callarias* L., in the Barents Sea, Trout (1957) suggested that the immature fish carry out a false spawning migration. 'The pattern of migration of the immatures is basically similar to that of the matures...with increasing age, the immatures' southerly winter migration approached in length that of the mature cod, as if, in the years immediately prior to maturity they were making a "dummy run" towards the spawning ground.' The seasonal migrations of both the adult and immature cod have been related to changes in the activity of the thyroid gland in this fish (Woodhead, 1959). During the spring of 1956 and 1957, collections of thyroid glands from immature cod were made at stations from Bear Island to the Norwegian coast. It was hoped that a study of these glands might demonstrate further the relationship of the activity of the thyroid gland to the migration of the fish.

The thyroid glands and gonads were taken from immature cod of 50–90 cm, and preserved for histological examination by routine methods (Woodhead, 1959). Samples were taken in the four areas between Bear Island and Røst Bank, south-west of the Lofoten Islands, shown in Fig. 1. The lengths of all immature cod caught in these areas were recorded.

RESULTS

Lengths of the immature cod

The data for the lengths of the immature cod caught at Bear Island and on the Norwegian coast are summarized in Fig. 2. (An insufficient number of immature cod were caught in Areas II and IV for the construction of a length distribution histogram.) In March the length distribution of the immature fish on the Norwegian coast had a mode between 70 to 80 cm, whereas the immature cod caught at Bear Island were smaller with a mode at 55 to 65 cm. These results substantiate Trout's hypothesis of a lengthy 'dummy run' by the oldest immature cod.

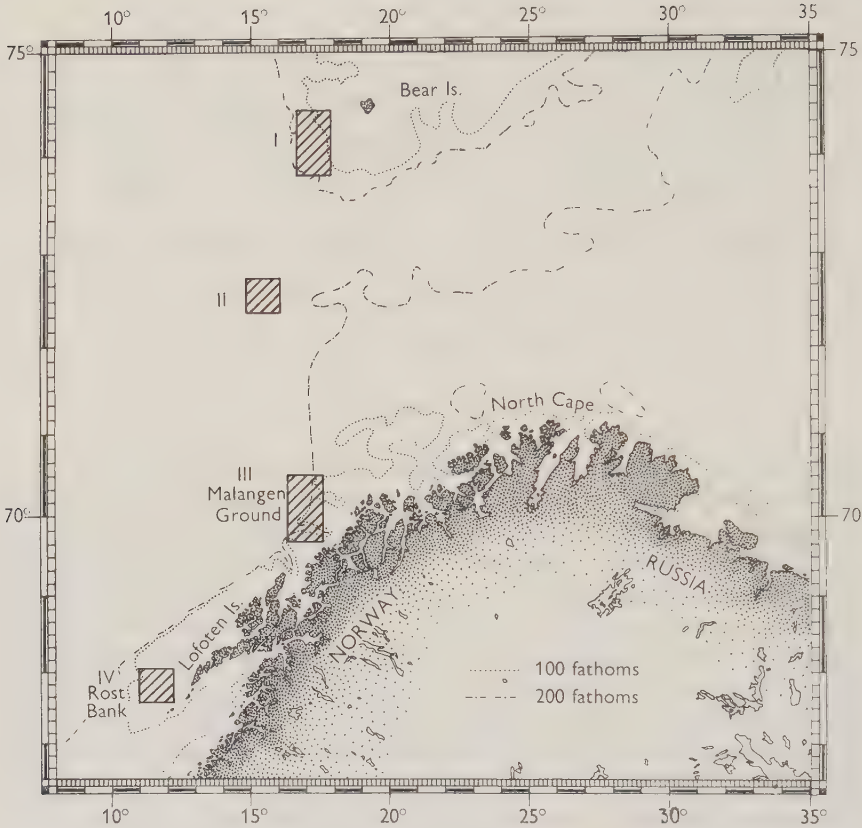

10°
15°
20°
25°
30°
35
75°
75
Bear Is.
I
II
North Cape
III
Malangen
Ground
70°
70°
RUSSIA
NORWAY
Lofoten Is.
IV
Rost
Bank
100 fathoms
200 fathoms
10°
15°
20°
25°
30°
35°

Fig. 1. Bear Island and the S.W. Barents Sea showing the four areas in which immature cod were caught in March 1957.

Thyroid activity

Thyroid glands taken from three immature fish caught on the Malangen ground (Area III) in March 1956 were found to have a significantly higher mean follicular cell height than those of a sample of ten immature cod caught at Bear Island (Area I) (Table 1).

Larger collections of material were made in the four areas shown in Fig. 1 during an extended investigation in March 1957. The activity of these thyroid glands, expressed as the mean follicular cell height for each sample of ten fish, is given in Table 2. The thyroids of the fish caught at Bear Island had the same level of activity as in the previous year, but the thyroids of the fish caught farther south were more active than those at Bear Island; thyroid activity tended to increase with the distance south of Bear Island at which the cod were caught (Fig. 3).

The thyroids of the immature cod caught in the deep water between Bear Island and the Norwegian coast (Area II) had a significantly higher level of activity ($p = 0{\cdot}02$) than those of the cod caught at Bear Island (Area I). The two groups of fish caught on the Norwegian coast also had significantly more active thyroids ($p < 0{\cdot}01$) than the Bear Island fish, but although the mean follicular cell height values increased in Areas II, III and IV the means were not significantly different (Table 3).

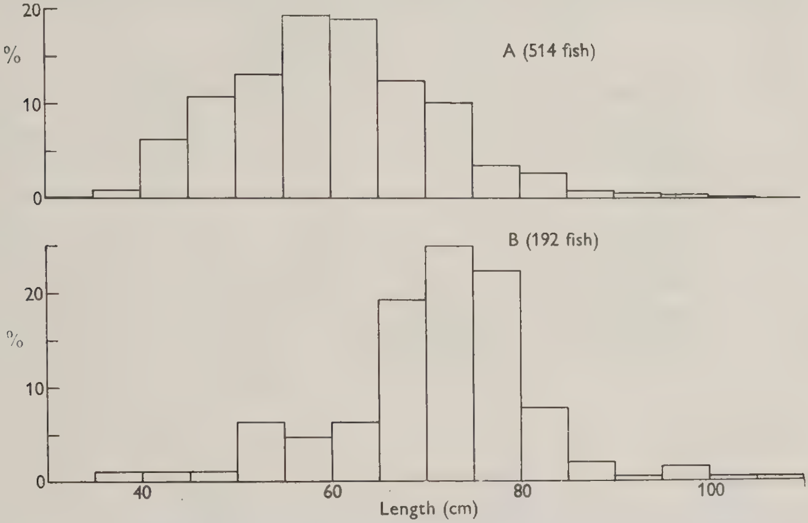


Fig. 2. The length distributions of the immature cod caught at (A) Bear Island and (B) on the Norwegian coast, in March 1957.

TABLE 1. MEAN FOLLICULAR CELL HEIGHT IN THE THYROID OF COD CAUGHT IN MARCH 1956

Sample	No. of fish	Mean follicular cell height (E.P.U.)	Standard deviation
Immatures Bear Island	10	55·1	3·2
Immatures Norway coast	3	68·7	6·8
Mature adults Norway coast	10	67·7	5·5

75 E.P.U. = 10 μ

DISCUSSION

The extent of the southerly overwintering migration of the immature cod from the feeding grounds on the Bear Island–Spitsbergen shelf increased as the fish became older, the largest immature cod migrating to the Norwegian coast, within a short distance from the spawning grounds of the adults. Examination of the thyroids of cod which had migrated farthest south showed that they were in a more active condition than those of the immature fish caught around Bear Island. In the Bear Island fish, which had reached their overwintering grounds

TABLE 2. MEAN FOLLICULAR CELL HEIGHT IN THE THYROID OF COD CAUGHT IN MARCH 1957

Sample	No. of fish	Mean follicular cell height (E.P.U.)	Standard deviation
Immatures Area I	10	54·3	4·5
Immatures Area II	10	61·9	7·8
Immatures Area III	10	64·4	5·4
Immatures Area IV	10	66·4	3·7
Mature adults Area III	10	67·4	6·3

75 E.P.U. = 10 μ

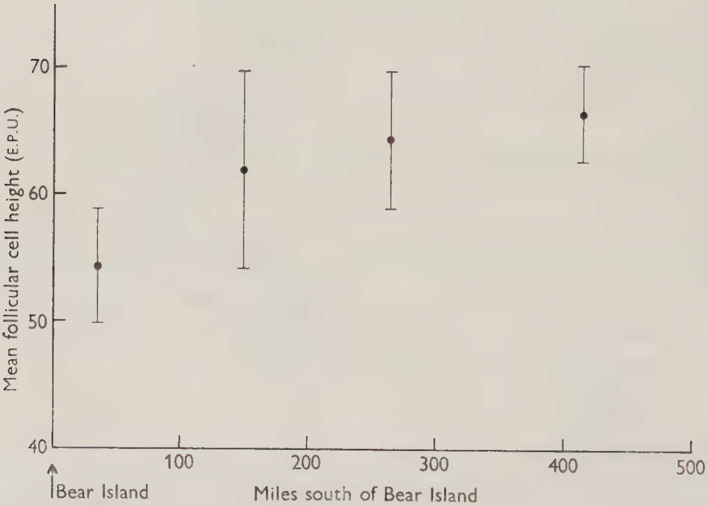


Fig. 3. The mean follicular cell height of the thyroid gland in immature cod caught in Areas I, II, III and IV, compared with the distance from Bear Island at which the fish were caught. The follicular cell height is expressed in eyepiece units (E.P.U.).

TABLE 3. COMPARISON OF THE MEAN FOLLICULAR CELL HEIGHT VALUES

Comparison between areas	Significance of difference (p)
I, II	0·02
II, III	0·4
III, IV	0·3
I, III	< 0·01
II, IV	0·1

around the edge of the Bear Island shelf, the mean follicular cell height had declined from a January level of 64·8 ± 8·7 to a level of 54·3 ± 4·5. The fish caught on the Norwegian coast still had a follicular cell height of 64·4 ± 5·4, which was not significantly different from the January level; indeed there was no significant difference between the levels of activity in the thyroids of immature cod in the October, November–December, January and the March–Norwegian coast samples. It appears that the thyroid cycle in the immature cod does not rise to a peak as in the adults (Woodhead, 1959), but that once the gland has become active, activity continues at a steady level with a mean follicular cell height of about 66·4 E.P.U. In the large immature cod thyroid activity continues over a more prolonged period than in the smaller immatures; this coincides with the increase in the length and duration of their overwintering migration, which in the largest immatures approaches in length the spawning migration of the adult cod. It seems that in the immature cod the active contranatant migration continues as long as the thyroid gland remains active. This evidence lends further support to the suggestion that the activity of the thyroid gland in the cod may initiate and sustain active and lengthy migrations.

Although the larger immature cod carry out a winter migration which is nearly as long as that of the mature adult cod, the cycle of activity in the thyroid gland of these fish is of a lesser order, although of the same duration as that in the mature cod. Pickford has suggested (Pickford & Atz, 1957) that a minimal level of thyroid activity may be necessary for gonad maturation and it may be that in the cod this is represented by the difference between the thyroid cycle of the adults and that of the large immature fish.

SUMMARY

The average length of the immature cod caught between Bear Island and the Norwegian coast in March 1956 increased from north to south and it appeared that the length of the southerly overwintering migration increased as the fish became older. In the fish caught on the Norwegian coast the thyroid gland was still fully active, whereas in the smaller fish to the north thyroid activity had declined considerably. It is suggested that the greater length of the migration in the larger immature cod is related to the continued high level of thyroid activity.

REFERENCES

Pickford, G. E. & Atz, J. W., 1957. *The Physiology of the Pituitary Gland of Fishes.* New York: Zool. Soc.

Trout, G. C., 1957. The Bear Island cod: migrations and movements. *Fish. Invest. Lond.*, Ser. 2, Vol. 21, No. 6. 51 pp.

Woodhead, A. D., 1959. Variations in the activity of the thyroid gland of the cod, *Gadus callarias* L., in relation to its migrations in the Barents Sea. I. Seasonal changes. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 407–15.

ADDENDUM

Since this work was submitted for publication, Swift (1959) has reported that in yearling brown trout the changes in the height of the thyroid follicular epithelium are inversely related to the temperature of the environment, and he interprets this as further evidence that the basic function of the thyroid is in the control of the animal's metabolism, in this case in such a fashion as to compensate for changes in the environmental temperature. Changes in environmental temperature cannot similarly explain the seasonal cycle of activity in the thyroid of the Barents Sea cod. The cod were found at the lowest temperatures (down to −0·5° C.) in shallow water on the Bear Island—Spitsbergen Shelf during the summer months, when thyroid activity was at a minimum. Highest water temperatures (5 to 6° C.) were encountered during mid-winter when the matures and the oldest of the immature cod were caught in warm water of Gulf Stream origin on the Norwegian coast; the thyroids of these fish were then maximally active. The comparison of thyroid glands taken from fish caught at different temperatures on the same cruise failed to show any significant differences in mean epithelial cell height, except during March when the immature cod around Bear Island at 1 to 2° C. had less active thyroids than those immatures at 5 to 6° C. on the Norwegian coast.

REFERENCE

Swift, D. R. (1959). Seasonal variation in the activity of the thyroid gland of yearling brown trout *Salmo trutta*. *J. exp. Biol.*, Vol. 35, pp. 120–25.

J. mar. biol. Ass. U.K. (1959) 38, 423–424
Printed in Great Britain

SEA SURFACE TEMPERATURES AT MILLPORT

By H. Barnes

The Marine Station, Millport, Scotland

Sea surface temperatures have been taken daily at Keppel Pier, Millport (55° 44′ 55″ N. lat.; 4° 54′ 20″ W. long.), for the past 10 years (1949–58), the data being included in returns to the Meteorological Office. The records are not given in their standard publication and they are, therefore, presented here to supplement those of Cooper (1958) for inshore waters at Plymouth some 400 miles farther to the south. For some years the samples were drawn in a specially constructed container fitted with a Fahrenheit thermometer which was read to the nearest whole degree; more recently the bucket method has been used, and a centigrade thermometer graduated in degrees and read to an estimated tenth of a degree. The two methods were checked against each other before the change was made and the results found to be consistent. All readings were taken at 09.00 h G.M.T., irrespective of the state of the tide. The results are shown in Table 1.

The form of the annual temperature regime is typical of boreal waters; there are, however, considerable variations from year to year, not only in the mean values but also in the temperature pattern and these variations are of ecological significance. For example, in a long cold spring the spawning of some species is delayed. In some years the temperature, after falling steadily during the autumn and winter, remains almost constant from January to April; e.g. in 1952 and 1953 when the value remained close to 7° C. during these months. In other years the temperature continues to fall during the early part of the year; in 1956, for example, this fall continued until late March, whereas in 1949 the temperature began to rise gradually in early March. In both 1954 and 1955 the temperature fell until early February, but whereas in 1954 this was followed by a slow rise, in 1955 the minimum was maintained throughout March. In 1950 the minimum was reached late in January.

Apart from day-to-day fluctuations due to local heating in shallow waters there are also, during the summer, periods of low temperature extending over 2–3 weeks; these may be the result of up-welling in the deeper basins and could be of considerable importance in the destruction of warmer water species carried into the area during the warmer months of the year.

REFERENCE

Cooper, L. H. N., 1958. Sea temperatures in Plymouth Sound. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 1–3.

TABLE 1. MEAN MONTHLY SEA SURFACE TEMPERATURES (° C.) FOR MILLPORT, 1949–58, TOGETHER WITH MAXIMA AND MINIMA ATTAINED IN EACH MONTH

	Jan.			Feb.			Mar.			Apr.			May			June		
Year	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.
1949	7·9	8·4	7·2	7·4	7·8	6·7	7·2	7·8	6·1	8·2	9·0	7·5	9·8	11·1	8·6	11·7	14·4	10·6
1950	8·1	9·1	6·4	7·1	7·7	5·5	7·6	8·4	6·9	8·2	8·8	7·6	9·2	10·2	8·1	12·0	13·4	9·7
1951	6·3	6·9	5·3	6·2	6·8	4·8	6·6	7·2	5·2	7·1	8·1	6·4	8·4	9·6	7·0	10·5	12·0	9·1
1952	7·4	8·4	6·7	7·1	8·0	5·9	7·3	7·9	6·8	8·1	8·9	7·0	9·3	10·8	7·9	10·6	12·9	9·0
1953	7·4	7·8	7·0	7·0	7·6	6·3	7·3	7·8	7·0	7·4	8·2	6·8	9·4	11·0	7·7	11·3	13·5	9·5
1954	8·7	9·9	7·2	7·0	7·6	6·4	6·5	7·0	6·0	7·5	8·2	7·0	8·7	10·1	7·5	11·0	12·3	9·8
1955	7·7	8·9	6·1	6·8	7·5	6·0	6·1	6·4	5·8	7·4	8·9	6·3	8·9	9·8	7·9	10·7	12·8	9·2
1956	7·5	8·4	6·8	6·3	6·7	5·9	6·2	6·9	5·8	7·1	7·9	6·5	9·0	11·7	7·9	10·3	11·3	9·3
1957	7·3	8·0	6·5	6·4	7·2	5·9	6·9	7·6	6·0	8·0	9·3	7·2	8·9	10·2	7·8	11·2	12·9	9·8
1958	7·2	8·0	6·4	6·4	7·0	6·0	5·9	6·5	5·2	6·3	7·3	5·5	8·1	9·8	7·1	9·7	11·4	8·4
10-year average	7·55	—	—	6·77	—	—	6·76	—	—	7·53	—	—	8·97	—	—	10·90	—	—

	July			Aug.			Sept.			Oct.			Nov.			Dec.			
Year	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Annual Mean
1949	12·7	14·2	11·4	13·1	15·0	13·1	14·3	15·3	13·4	13·2	13·9	11·4	11·0	12·0	10·0	9·2	10·7	8·2	10·48
1950	14·0	15·5	12·1	14·7	15·3	13·8	13·4	14·2	12·2	11·7	12·5	10·7	10·3	11·2	9·0	8·1	9·7	6·6	10·37
1951	12·5	14·0	10·9	13·1	14·8	11·1	12·9	13·5	12·2	12·0	12·9	10·8	10·7	11·5	9·7	8·9	10·1	7·4	9·59
1952	12·9	14·6	11·2	13·5	15·1	12·6	12·4	13·6	11·7	11·2	12·2	10·4	10·0	10·7	9·2	8·4	9·7	7·2	9·85
1953	13·1	14·2	11·6	14·3	15·0	13·5	13·5	14·1	13·0	12·6	13·9	11·7	10·6	11·8	9·8	9·9	10·8	9·2	10·31
1954	12·4	14·0	11·3	13·2	14·8	11·9	12·8	14·0	11·5	11·5	12·1	9·8	9·9	10·8	8·6	9·1	9·9	8·3	9·86
1955	12·8	14·7	10·5	13·6	15·1	12·3	13·5	14·5	12·9	11·8	13·0	10·8	10·6	11·0	9·9	9·0	10·0	7·8	9·91
1956	12·6	13·3	12·0	12·3	12·8	11·7	12·1	12·5	11·7	11·5	12·2	10·4	10·4	11·0	9·3	8·6	9·8	6·9	9·49
1957	12·5	13·7	11·2	12·9	13·9	12·3	12·3	13·0	11·4	11·5	12·2	10·5	10·2	10·7	9·3	8·9	9·9	7·9	9·75
1958	11·8	13·7	9·9	13·2	14·6	11·7	13·2	14·5	12·2	11·8	12·8	11·0	10·8	11·5	9·9	9·5	10·9	8·4	9·49
10-year average	12·73	—	—	13·39	—	—	13·04	—	—	11·88	—	—	10·45	—	—	8·96	—	—	9·91

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

Atkins, D., 1958. A new species and genus of Kraussinidae (Brachiopoda) with a note on feeding. *Proc. zool. Soc. Lond.*, Vol. 131, pp. 559–81.

A new species and genus, *Pumilus antiquatus*, of kraussinid brachiopod from shallow water, Lyttelton, New Zealand, is characterized by the possession of a schizolophous lophophore in the adult, supported by both divergent lamellae and spicules: the shell is smooth, less than 5 mm long and strongly sulcate. The gonad is hermaphrodite.

Pumilus is placed in the Kraussinidae for the following reasons: (1) the brachial skeleton resembles that of immature *Kraussina rubra*; (2) the presence of spicules in the lophophore; (3) similar arrangement of muscles to that in *Megerlia*.

The ciliary feeding mechanism is briefly described. D.A.

Bone, Q., 1958. Synaptic relations in the atrial nervous system of amphioxus. *Quart. J. micr. Sci.*, Vol. 99, pp. 243–61.

The system of nerve-cells upon the gut and diverticulum of amphioxus (*Branchiostoma*) is described in detail; degeneration experiments were performed showing that these cells connect with the central nervous system by their own axons, running in the dorsal root nerves. Special attention is paid to the problems of the multi-nucleate nerve cells in the plexus, and to the possibility of asynaptic connexion between neighbouring nerve cells. No sheath cells have been observed upon the peripheral nerve fibres, either within the atrial plexus or upon the dorsal root nerve bundles.

It is suggested that the atrial system of nerve cells arises in ontogeny by emigration of cell bodies from the central nervous system; evidence is put forward supporting this suggestion.

The relation of the amphioxus visceral plexus to similar plexuses in craniates is discussed, and it is concluded that the system is not homologous with the enteric systems of nerve cells in the vertebrates. Q.B.

Brunet, P. C. J. & Carlisle, D. B., 1958. Chitin in Pogonophora. *Nature, Lond.*, Vol. 182, p. 1689.

The tubes of three species of *Siboglinum* and of one species of *Zenkevitchiana* consist of protein and chitin as determined by the chitosan iodine test. Acid hydrolysis of the tubes yields about 10 amino acids and D-glucosamine, while enzymic degradation yields *N*-acetyl-D-glucosamine.

No trace of glucose was found in the hydrolysates, thus discounting the suggestion that the tubes consisted of cellulose. D.B.C.

Hill, A. V. & Howarth, J. V., 1958. The initial heat production of stimulated nerve. *Proc. roy. Soc.*, B, Vol. 149, pp. 167–75.

The work described was an extension of research previously done at the Plymouth Laboratory (Abstract in this *Journal*, Vol. 37, p. 807). In this it was shown that a single impulse in a crab's nerve at 0° C. is accompanied by a brief heat production followed immediately by an absorption of heat. The present paper shows records of heat production during repetitive stimulation at different frequencies at 0° C. With 5 shocks/s the diphasic character of the heat production was very clearly shown. With 10 shocks/s the instruments were too slow to show it. At all frequencies the experimental records were identical with the corresponding curves calculated from a knowledge of the deflection produced by a single impulse. Above 12° C. the instrument could not resolve the diphasic heat production at any frequency.

Similar experiments were made at University College London using medullated nerve of the frog. There was no evidence of diphasic character in the heat production, but this could be due to the instruments being too slow to detect it. Analysis of records for repetitive stimulation at higher frequency located the 'initial heat' within 0·04 s of the stimulus. J.V.H.

Potts, W. T. W., 1958. The inorganic and amino-acid composition of some lamellibranch muscles. *J. exp. Biol.*, Vol. 35, pp. 749–64.

The sodium, potassium, and chloride content and the inulin spaces of a number of lamellibranch muscles have been measured and the intracellular concentrations of the ions calculated. The muscles examined were the large striated part and the slowest part of *Pecten* adductor, the fast and slow parts of *Mytilus* adductor and the anterior byssus retractor of *Mytilus*, the fast and slow parts of *Anodonta* adductors, and the ventricles of *Mytilus* and *Anodonta*.

The fast striated portion of *Pecten* adductor is similar in ionic composition to other striated muscles or to nerve. The intracellular concentrations of sodium and chloride are low and the potassium and chloride ions are approximately in Donnan equilibrium with the blood. The slower muscles contain much greater quantities of sodium and chloride ions and the potassium and chloride ions are not in Donnan equilibrium with the blood. The divergence from the equilibrium condition is greatest in the slowest muscles. The possible significance of these results is discussed and it is concluded that it is probable that both the potassium and the sodium ions are actively transported in the slow muscles of lamellibranch.

Analyses have also been made of the phosphate compounds and free amino acids in *Mytilus* and *Anodonta* muscles. *Anodonta* muscles contain about half the concentration of phosphate compounds that *Mytilus* muscles contain but only about 4% as much amino acids.

Mytilus muscles adapt to a 50% fall in blood concentration in part by osmotic swelling of the cells, in part by the loss of free amino acids. *Anodonta* muscles adapt to an increased concentration by the shrinkage of the muscle fibres, an increase in the sodium and chloride concentration in the cells and by an increase in the free amino acid content of the cells. In both *Mytilus* and *Anodonta* the potassium and phosphate content per cell remains relatively constant during osmotic changes. W.T.W.P.

BOOK REVIEWS

OCEANOGRAPHY AND MARINE BIOLOGY: A BOOK OF TECHNIQUES

By H. BARNES

Published by George Allen and Unwin, Ltd., 1959. Pp. 218; illustrations 110. Price 35*s*.

The techniques described here come under four headings, viz.: Sampling the Living Organisms, The Use of Sound Waves, Some Properties of the Water itself, and Photography and Television. Those described in the first section include bacteriological sampling, plankton catching, sampling bottom fauna with trawls and dredges, and the taking of bottom samples with grabs and corers. The section on the properties of the water is concerned with temperature measurement, collection of samples, and drift and current measurements. The other two sections are up-to-date accounts of acoustic and optical methods of underwater exploration. Dr Barnes is known to be widely experienced in marine investigations and he writes with authority on underwater television. His book is readable and well arranged, and the descriptions of apparatus are accurate and lucid. The line illustrations are neat, and judiciously simplified, and the photographs help out the text. There are nearly 140 references to original sources, and an adequate index.

The book should interest the mechanically minded general reader, the student, and the newcomer to marine biology who wishes to know what can be done at sea and how it is done. Those with more experience may be disappointed that it is descriptive rather than critical of the instruments described, and that it is in no way a manual for their use.

F.A.J.A.

MARINE ECOLOGY

By HILARY B. MOORE

Published by John Wiley and Sons, Inc., New York (London: Chapman and Hall, Ltd.), 1958. Pp. i–xi, + 1–493, with 244 text-figures. Price 76*s*.

Dr Moore is to be congratulated on having brought together in one volume the extremely varied studies that collectively are called marine ecology. In the limited compass of a standard text-book he reviews what is known of the deep sea and the world of plankton, as well as of the specialized conditions of the intertidal zone, and pays especial attention to such perennially interesting topics as geographical distribution, vertical migration and coral reefs. The book, which is lavishly provided with tables and text-figures, is divided into three parts; the first two describe the physical environment and the various habitats available to living organisms, while the last deals with the organisms themselves. There is a large bibliography, which has been restricted, however, to references likely to be consulted by English-speaking students. This means that there are few references on the deep sea, a field in which Russian workers are now very active. A corresponding gap in the text has been filled with underwater photographs of doubtful value.

This volume provides an admirable source-book for American-style advanced courses: by judicious selection from the great detail presented, it would also serve for undergraduate studies in marine biology.

A.J.S.

Molluscs

By J. E. Morton

Hutchinson and Co. Ltd., London, 1958. 232 pp. Price 10*s*. 6*d*.

This book is an introduction to the wide field of what is now known as 'functional morphology', of the molluscs. Much original work on this subject has been published in recent years by workers in this country, notably by Yonge, Graham, and Fretter, and the author has presented his subject in an eminently readable style. Chapters cover external form and habits, the main organ systems, nervous systems and behaviour, and the classification and evolution of the three main classes. This is not a text-book of anatomy, and morphology has been treated throughout in its functional and evolutionary contexts. The ecological aspects of the phylum, well covered in other works, are necessarily condensed. Published as a compact volume at a very reasonable price, this book should appeal as a source of reference to all workers concerned with the Mollusca.

N.A.H.

The Underwater Guide to Marine Life

By Carleton Ray and Elgin Ciampi

First published, in 1956, by A. S. Barnes and Company Inc., New York. Published in the British Commonwealth in 1958 by Nicholas Kaye Ltd., London. Printed in the U.S.A. Pp. xiii + 338 + 16 plates and 194 text-figs. Price 30*s*.

This most attractively illustrated book was written to aid the underwater swimmer with little or no knowledge of marine life. Apart from minor errors it does on the whole succeed in giving a sound introduction to the subject, and it will enable swimmers in American waters to recognize and know something about most of the living things they are likely to meet below the surface. It must be emphasized that this is indeed an American book and it is of very limited value to divers in European seas. The species described are all from the two coasts of the United States, a large proportion of them from tropical and semi-tropical regions; few of them are to be found in Europe. In view of its purpose the larger organisms, especially the fishes, are dealt with at greatest length, the invertebrates and plant life occupying a much smaller part of the book. The sections on these could well have been expanded at the expense of the chapters on under water diving and photography, which are too short to be of real value. The numerous drawings by a Japanese artist are very pleasing; many of them are adapted from standard works. The coloured drawings are occasionally disappointing; the figure of the garibaldi, for instance, is not as brilliantly orange as it should be, and one wonders whether any octopus was ever such a grass-green as in the figure in this book. There are some excellent underwater photographs in monochrome and in colour: some European marine biologists will probably like to possess the book for these and for the general overall, though incomplete picture (heavily biased in favour of the fishes) it gives of American marine life.

D. P. W.

J. mar. biol. Ass. U.K. (1959) 38, 429–437
Printed in Great Britain

THE FURTHER SPREAD OF *ELMINIUS MODESTUS* IN THE BRITISH ISLES TO 1959

By D. J. Crisp and A. J. Southward

Marine Biology Station, Menai Bridge, Anglesey and The Plymouth Laboratory

(Text-fig. 1)

Since the surveys described in our last report (Crisp, 1958) on the distribution in north-west Europe of the immigrant Australasian barnacle, *Elminius modestus* Darwin, the species has become much commoner along the western coasts and has finally spread to Ireland (Beard, 1957). In this report we present the results of surveys made in 1958–9 along the eastern side of the Irish Channel and in the Isle of Man, as well as full records of a survey of the Irish coast in 1958. The methods employed were similar to those described previously (Crisp, 1958; Bishop & Crisp, 1958).

We are indebted to the Browne Research Fund of the Royal Society and to the Marine Biological Association for financial assistance. Dr J. R. Lewis and Mr W. J. Ballantine have kindly supplied additional information for Ireland.

EASTERN SIDE OF THE IRISH CHANNEL

The changes in distribution of *Elminius* along the west coast of England and Wales have been described up to 1956 (Crisp, 1958). At that time an extension was just beginning around the Lleyn peninsula into the north end of Cardigan Bay. Between 1956 and 1958 the species spread southward over most of the north end of the bay; but, except for the area between Pwlheli and Barmouth, where heavy settlements occurred, it was otherwise confined to harbours and estuarine areas (Fig. 1).

At the south end of Cardigan Bay a relatively local settlement was discovered around the port of Fishguard. This colony, and a smaller one in the harbour at Solva, are believed to have originated from Milford Haven, where *Elminius* has been common for 10 years. The slow spread from this direction, in comparison with the rapid extension from the north, must be due to the lack of suitable habitats for the species along the wave-beaten Pembroke coast and the probable presence of adverse currents inshore. Similar factors may have prevented the spread of the species around the Lizard and Lands End in west Cornwall.

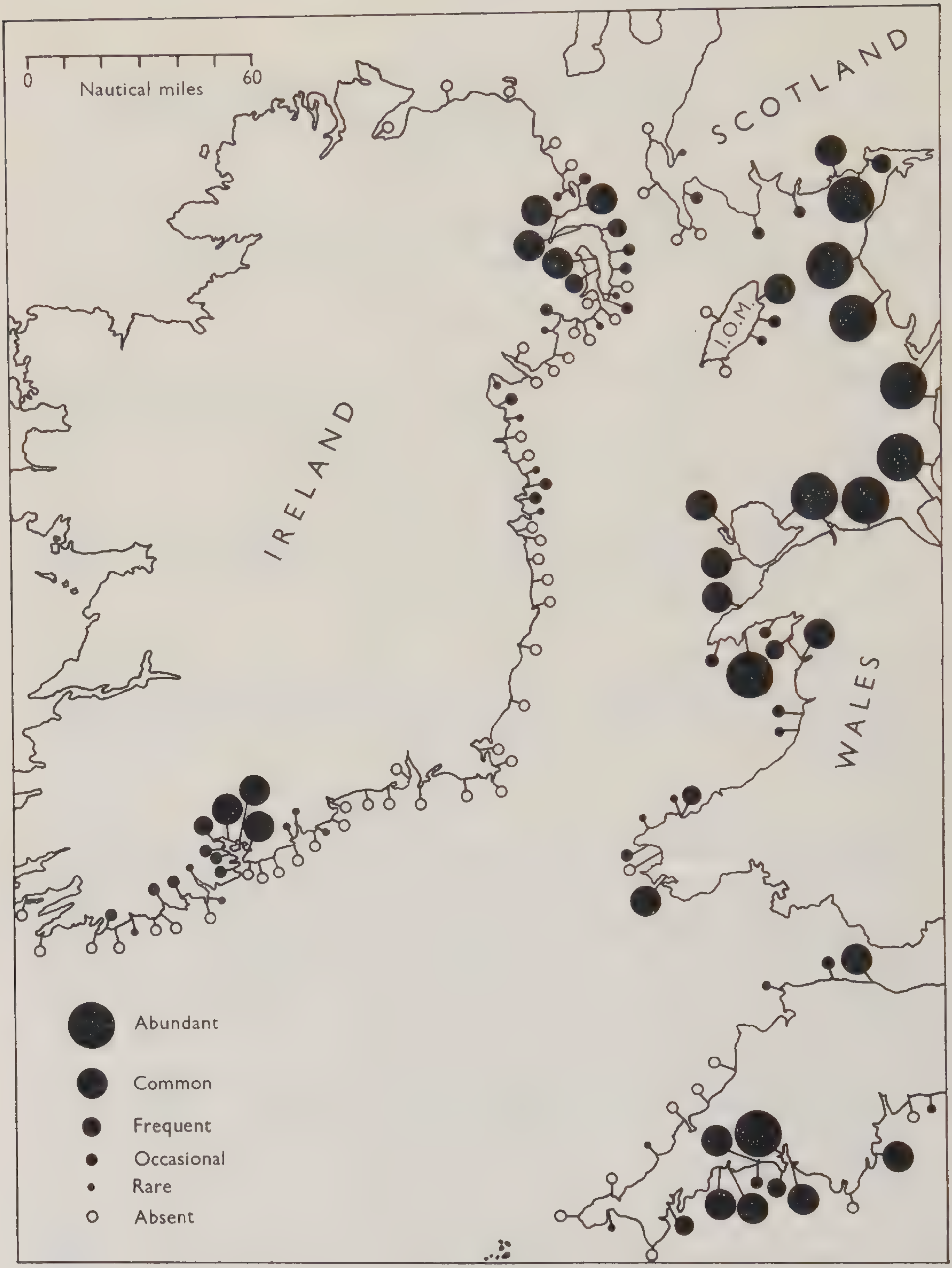

0 Nautical miles 60
SCOTLAND
I.O.M.
IRELAND
WALES
Abundant
Common
Frequent
Occasional
Rare
Absent

Fig. 1. The distribution of *Elminius modestus* in 1958–9. Stations visited in Ireland in 1958 are shown in full only for the east and south coasts. Isle of Man records made in 1959, those on west coast of Britain in 1958 or 1959. In localities where the species is common or abundant not all stations can be shown. For quantitative meaning of symbols refer to Table 1.

Outside the Bristol Channel, *Elminius* has, so far, failed to establish itself on the exposed coasts of north Devon and Cornwall, or even in Mounts Bay. Along the south Devon and Cornish coasts, from the Exe to the Helford estuaries, *Elminius* is present, and often abundant, in most of the harbours, and since our last report has been slowly increasing on the wave-beaten open coast. West of the Lizard, however, the previously reported settlement at Penzance appears to have failed: only a few old specimens could be found there in 1959, and the filling in of the inner harbour appears to have removed the only suitable habitat for *Elminius* at that end of Mounts Bay. The earlier record (rare) for St Ives in 1955 (Crisp, 1958) has not been confirmed, in spite of extensive searches, and since the specimen was not kept, we cannot now be sure whether this was a true sporadic settlement or a mis-identification of a distorted example of *Balanus balanoides*. Genuine sporadic settlements seem to have occurred in the Newquay area in 1958, when one individual was seen on the open coast, and in 1959, when another was found in the harbour. The species was not seen elsewhere along this coast, and it would be an idle speculation to discuss the probable origin of such few specimens.

Along the northern part of the Irish Channel there has been little further extension of range. *Elminius* is, as in 1956, abundant in the Solway Firth and common in Wigtown Bay. It is now present on wave-beaten promontories such as Burrow Head and Meikle Ross, but is still relatively rare in Luce Bay and has not yet passed the Mull of Galloway. Similarly, there has been little change in the isolated and sparse colony in Lough Ryan at the entrance to the Clyde Sea.

ISLE OF MAN

Except at Ramsey, where the species has become more common, there has been little change in the distribution and abundance of *Elminius* in the Isle of Man (Fig. 1). It is present in small numbers in the harbour at Laxey, south of Ramsey, and one specimen was found at Douglas after more than 1 h searching of suitable habitats, but is absent from the west and south coasts of the Island. The apparent failure of *Elminius* to spread southward from Ramsey must be due to the local hydrography. The residual flow through the Irish Sea is northward, and the set of the tidal currents is such that there is little chance of larvae being carried direct from the north end of the island to the south.

IRELAND

An earlier survey of the entire coast of Ireland was made in 1952, and further observation in 1953 between Lough Foyle and Dublin showed that *Elminius* had not then been able to colonize Ireland (Crisp & Southward, 1953; Southward & Crisp, 1954). Its failure to reach Ireland was attributed to the isolation of the coast from the mainland of Britain by the wide channel of deep water, which prevented normal dissemination of the planktonic larvae by water-currents. In 1957, however, *Elminius* was reported to be present in small numbers outside Lough Ine, Co. Cork (Beard, 1957), while we were planning another survey. The new survey was made over the whole coast, as well as in the neighbourhood of this record, particular attention being paid to the eastern coast which might be vulnerable to direct infection from the stronger populations now present on the opposite side of the Irish Channel (see p. 429).

Full details of localities where *Elminius* was found in Ireland in 1958 are given in Table 1; the distribution on both sides of the Irish Channel is shown in Fig. 1. The whole of the north and west coast, from Glenarm to Bantry Bay, was apparently free of *Elminius*, even in the vicinity of the ports of Londonderry and Galway. On the south and east coasts there were several distinct regions where *Elminius* was found. The most northerly colonization extended from Larne Harbour to Newcastle, Co. Down; dense populations were found in Belfast Lough and Strangford Lough, and in places reached one individual per cm^2 of surface.

South of this strong entrenchment there were two thinly settled patches, one from Dundalk to Port Oriel, another from Skerries to Howth. These settlements nowhere exceeded 100 per m^2 and the density was often much less. Farther south along the east coast we did not find any more specimens of *Elminius*, not even in the busy harbours at Dun Laoghaire, Wicklow, Arklow and Wexford, nor in the estuary at Waterford.

The remaining area in which *Elminius* was found corresponded to that first reported by Beard (1957). The settlement apparently extended from Ardmore to Lough Ine, but the species was common only in the sheltered bays and channels of Cork harbour. Here, in favourable places such as Rathcoursey, populations up to one individual per cm^2 were sometimes found, with spat settling at the rate of 3 per cm^2, but over most of this large enclosed area the density did not exceed 0·1 per cm^2. No specimens were found at any of the wave-beaten open coast stations in this region.

The origin of the Elminius *colonization of Ireland*

Before discussing the way *Elminius* spread to Ireland it is worth while considering how suitable the Irish coast is for the establishment of the species. By analogy with the known distribution of *Elminius* in other parts of Europe (see Den Hartog, 1953; Crisp, 1958; Bishop & Crisp, 1958), most of the north and west coasts would be expected to be as unfavourable for the species as are the equally wave-beaten coasts of Cornwall and Brittany. The larger estuaries and bays, such as Lough Foyle, the River Shannon and Bantry Bay should eventually support isolated populations similar to those of the drowned valley systems of Cornwall and Brittany. The south-east and east coasts of Ireland, which are sheltered from swell and the prevailing south-westerly winds might be expected to be more favourable to *Elminius*, but these shores have nowhere the drowned alluvial coast, termed 'côtes à estuaires' by de Martonne (1935), which in south-east England and the Netherlands is so particularly favourable to the species. The stretch of coast from Wicklow to Wexford comes nearest to these conditions, but the sand and gravel shores are subject to severe scouring, the tidal range is small and the shore fauna as

TABLE 1. DISTRIBUTION OF *ELMINIUS MODESTUS* IN IRELAND, 1958

Observations were made between May and September: many negative records are omitted here, as are the whole of the stations on the north and west coasts where the species was not found.

A Density over 1 per cm^2, covering 30% or more of available area.
C Density from 1 to 0·1 per cm^2, most specimens close enough to breed.
F Density, from 0·1 to 0·01 per cm^2, some of them close enough to breed.
O Density 100 to 1 per m^2; often local and needing to be searched for; rarely close enough to breed.
R Less than 1 per m^2; only a few isolated specimens in a 1 h search.
N None found in 1 h search in suitable place.

Place	Shore*	Abundance
Larne, coast road	R, B, I	N
Larne Harbour	P, B, M	R
Port Muck	RIE (chalk), B	O
Whitehead	P, B, G,	C
Carrickfergus	R, P, B, S, M	C (spat A)
Bangor Harbour	P, S, M	C
Bangor	RI	F
Donaghadee, north end	RI, B	F
Donaghadee Harbour	P, S, M	F
Donaghadee, south	RE, B	N
Ballywalter	P, S, M	O–R
Burr Pt., Ballyhalbert	RE, B, S	O–R
Kearney Pt.	RSIE	N
Ballyquintin Pt.	B	O–R
Portaferry	B, S, M	R
Kirkubbin	P, B, S, M	F (spat A)
Greyabbey	B, S	C
Kilclief	R, S	N
Ballyhornan	RS, G	N
Ardglass Harbour	P, M	R
St John's Pt.	RSE, B	N
Rossglass	R, B, S	N
Dundrum	B, G, S, M	O–R
Newcastle	R, B, S	R
Annalong	R, P, B	N
Clashbridge	RS, B	N
Rostrevor	P, B, M	N
Greenore Pt	P, B, S	N
Dundalk Harbour	P, B, S, M	R (1 only)
Annagassan	B, S	O–R
Port Oriel	R, P, B	R
Baltray	P, M	N
Balbriggan	R, B, S	N
Skerries	RI, B	R
Rush	P, S	O–R
Malahide	R, S, M	O–R
Howth	R, P, G	R
Dun Laoghaire	R, P	N
Dalkey	R, B, G	N
Ardmore	RE	R
Ardmore Head	RS	N
Youghal, bridge	P	R
Pilmore, bridge	P	R
Knockadoon Head	RS	N
Ballycotton	RIE, P	N
Power Head	RS	N
Inch Strand	R	N
Gyleen	RE, B	N
Farsid, nr. Aghada	RI, P, G, S, M	C–F (little spat)
Cobh	P	F
Rathcoursey	P, B, G, M	C (spat A)
Ballynacorra	B, M (fresh water)	N
Belvelly	P, B, G, M	C (spat C)
Cobh Junction	B, M	O–R
Cork	P	F
Passage West	RS, M	O(W.J.B.)
Monkstown	B, G, M	O(W.J.B.)
Myrtleville	RSI	O(W.J.B.)
Kinsale Harbour	P	R
Oldhead Harbour Kinsale	P, B, G	R
Courtmacsherry	B, G, M	O
Clonakilty, North Ring	P	O–R
Inchydoney	P	N
Dunowen	RIE	N
Ballinglanna	RIE, B, G, S	N
Galley Head	RSI	N
Glandore Harbour	P, M	O
Toe Head	RSI	N
Toe Head Bay	RIE, B	N
Tragumna	RSI, B	N
Barloge Creek, harbour	P, B, G	O–R
Baltimore	R, P, B, G, M	N
Berehaven	R, B, G	N

W.J.B.: records by Mr W. J. Ballantine.

* R, rocks (S, steep; I, irregular; E, extensive); B, boulders; G, gravel; S, sand; M, mud; P, artificial substrata.

a whole is impoverished (Southward & Crisp, 1954). Under such conditions it is unlikely that *Elminius* would be common on the open coast, although it would be expected to be present in areas of sheltered water such as at Rosslare and Wexford. Apart from the loughs and estuaries, the region of the east coast around Dundrum and Dundalk bays would appear to be the most favourable in Ireland to *Elminius*. Wave action is less and the extensive bays and areas of shallow water should result in local warming-up during the summer and hence increase breeding and settlement of the species (Southward & Crisp, 1954; Crisp & Davies, 1955; Crisp, 1958).

In addition to suitability of the coast we must consider its accessibility to invasion by the two possible means of dispersal: marginal dispersal by larvae liberated into the sea and carried by currents, and remote dispersal, by adults carried on ships, shellfish and floating objects later liberating larvae when in harbour or washed up on the shore (see Crisp. 1958). The nearest accessible point on the Irish coast is Co. Down, which lies some 40 miles from the populations of *Elminius* in Wigtown Bay and the Isle of Man. The Co. Dublin coast is about 60 miles from the population in Anglesey. These distances exceed, though not greatly, the apparent minimum critical distance for colonization by *Elminius* across a sea barrier in British Waters (Crisp & Southward, 1953). However, the northward flow through the Irish Sea passes to the east of the Isle of Man, and thus close to the dense populations of *Elminius* on that side of the channel, before travelling through the North Channel. Although, in the earlier parts of the year, at least (Williamson, 1956), the northward flow is separated from the Antrim and Down coasts by a tongue of southgoing cold water, the possibility of colonization of this coast by marginal dispersal cannot, therefore, be ruled out.

Remote dispersal by ships carrying the adults depends on the likelihood of the infected ships remaining long enough in port to liberate larvae, and of the port being in an area suitable for the retention of larvae during development and for their later settlement. Belfast, lying at the head of a suitable lough, and frequently visited by ships of all kinds from across the Irish Sea, is a likely port of entry, as to a lesser degree are the ports of Londonderry, Larne, Wexford, Waterford and Cork. The ports of Dublin, Dun Laoghaire, Wicklow and Arklow, though likely to receive infected shipping, are less suitable for the establishment of *Elminius*.

The probable history of the colonization

All stretches of coast where *Elminius* was found in 1958 were situated on the more favourable east and south coasts, and two were clearly centred on the ports of Belfast and Cork. Remote dispersal is therefore indicated.

The population on the north-east coast may in fact be presumed to have been introduced by ships in, or close to, the port of Belfast. Within the lough, the size-groups of adult barnacles indicated an initial settlement prior to 1957.

The other dense population, in this region, in Strangford Lough, may be presumed to have been colonized by marginal dispersal from Belfast, and subsequently increased within the lough. If we assume that the open coast and the other loughs were also settled by marginal dispersal from Belfast Lough, the maximum spread northwards has been little more than 10 miles, whereas to the south it has extended to Newcastle, some 40 miles down the coast. This relative failure of the species to extend northwards may be ascribed to the presence of the south-going current along the Antrim and Down coasts. In addition, the region north of Belfast Lough experiences lower sea temperatures which would discourage an animal such as *Elminius* which depends on high summer temperatures for rapid breeding (Crisp & Davies, 1955). In contrast, conditions south of Strangford Lough would appear much more suitable for *Elminius*, as for the several southern forms that occur there in some abundance (Southward & Crisp, 1954). In general, the whole of the Antrim coast north of Belfast Lough seems unsuitable for barnacles; the native species are often sparse, and may rarely be present at all on the predominantly chalk rocks. It is worth noting that similar factors to these, viz. south-going currents, lower temperatures and less favourable substrata, are believed to have prevented the extension of *Elminius* north of the Humber on the east coast of England since 1950 (Crisp, 1958).

The areas of colonization lying between the Belfast settlement and Dublin Bay are smaller and more thinly populated than the other Irish populations. The main reason for regarding them as distinct from Belfast centre is that no trace of settlement was found in Carlingford Lough. This lough appears to offer as suitable an environment as Belfast Lough or Strangford Lough, and it is difficult to believe that marginal dispersal along the coast could have reached Dundalk without some larvae entering Carlingford Lough and establishing themselves there. Similar, but less cogent, arguments can be applied to the gap separating the two minor settlements, for the mouth of the River Boyne and the port of Balbriggan would seem to be no less suitable than the settled harbours of Port Oriel and Skerries on either side. In both areas of settlement the population density was so low that few, if any, individuals could have been capable of breeding. It is therefore difficult to account for these settlements unless there exists one or more centres of dispersal not discovered during the survey, containing individuals close enough to breed. There is a distinct possibility that these sparse settlements might have been derived from numbers of small craft which had become infected in other ports, and one such vessel, carrying many adult specimens was indeed observed at the quayside in Howth, where *Elminius* was scarce. The difficulty in accepting this possibility of multiple dissemination is that *Elminius* was entirely absent south of Howth. The harbours at Dun Laoghaire, Bray, Wicklow and Arklow would be expected to receive as many infected craft as the smaller and more exposed harbours of Port Oriel and Skerries. Further investigations after the passage of time may allow the correct interpretation to be chosen from these alternatives.

The most widespread settlement of *Elminius* in Ireland, that in the south-west, shows every sign of having arisen from an initial introduction somewhere in the sheltered channels and inlets of Cork harbour. From this area it has extended over 40 miles to the west but little more than 20 miles eastward: here again we have the possibility of a coastal current, this time from the east, influencing the dispersal of larvae. On account of the exposed nature of the coast, the spread has been confined to enclosed bays and harbours, as on the Cornish coast.

In the case of the Cork settlement, we are fortunate in having two means of estimating, albeit very approximately, the probable date of introduction of *Elminius*. The rate of spread along a coast of similar type, that of south Devon and Cornwall, is of the order of 10–15 miles a year (Crisp, 1958). Measuring from the Lough Ine and Glandore settlements, this would place the original entry some time in 1955. Further evidence was obtained from settlements present on piles which had been removed from the estuary just downstream of Cork, and were lying on the bank. According to local workmen the piles had been removed at various times, some that year, some in 1957, and some as early as 1956, but unfortunately they had not been kept separate. The oldest looking piles had lost all growth, but others had a mixed population of *Balanus improvisus* and *Elminius modestus* still attached, the latter at densities of up to 0·03 per cm^2. Many of the *Elminius* measured 9–10 mm diameter, and must therefore have settled in 1957, probably quite early in the season, and certainly in considerable numbers. This evidence suggests that the original settlement must have been present as long ago as 1956, and possibly earlier. *Elminius* was absent (or present in numbers too small to be revealed by survey) in 1952 when we inspected several stations in Cork harbour, and in 1953 when Dr J. R. Lewis examined the same area. From the two independent estimates, and the negative records, it seems reasonably probable that *Elminius* was established between 1954 and 1956. This original population must have been introduced by remote dispersal, since Cork harbour is far removed from any other locality where *Elminius* is plentiful.

SUMMARY

Since 1956, *Elminius modestus*, the immigrant Australasian barnacle, has increased its range and abundance along the west coast of Britain. At the same time it has established itself in Ireland, and is now common in two areas, centred on Belfast and Cork, respectively. Both areas have been independently colonized since 1953, the former probably and the latter certainly by remote dispersal on ships. Two further sparse settlements were discovered on

the east coast of Ireland from Dundalk to Howth, but further evidence is needed to determine whether these finds represent a southerly extension of the Belfast population, or independent invasions.

REFERENCES

BEARD, D. M., 1957. Occurrence of *Elminius modestus* Darwin in Ireland. *Nature, Lond.*, Vol. 180, p. 1145.

BISHOP, M. W. H. & CRISP, D. J., 1958. The distribution of *Elminius modestus* Darwin in France. *Proc. zool. Soc. Lond.*, Vol. 131, pp. 109–34.

CRISP, D. J., 1958. The spread of *Elminius modestus* Darwin in north-west Europe. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 483–520.

CRISP, D. J. & DAVIES, P. A., 1955. Observations *in vivo* on the breeding of *Elminius modestus* grown on glass slides. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 357–80.

CRISP, D. J. & SOUTHWARD, A. J., 1953. Isolation of intertidal animals by sea barriers. *Nature, Lond.*, Vol. 172, p. 208.

DEN HARTOG, C., 1953. Immigration, dissemination, and ecology of *Elminius modestus* Darwin in the North Sea, especially along the Dutch coast. *Beaufortia*, Vol. 4, pp. 9–20.

MARTONNE, E. DE, 1935. *Traité de Géographie Physique.* Paris.

SOUTHWARD, A. J. & CRISP, D. J., 1954. The distribution of certain intertidal animals around the Irish Coast. *Proc. R. Irish Acad.*, Vol. 57, Sect. B, No. 1, 29 pp.

Note added in proof

One of us (D.J.C.) was able to re-examine part of the north and east coasts of Ireland during a brief visit in July 1959, and *Elminius* was found at the following stations (to be added to Table 1):

Londonderry, main channel in L. Foyle	R, P, M	R
Dublin, North Wall	P, M	O–F

The species seemed well-established at Dublin, with a fresh spatfall of 2–3 per cm^2; this confirms the existence of a separate invasion of the Co. Dublin area. The record for L. Foyle cannot be regarded as establishing the species in that area. Only three old specimens of *Elminius* were found, all on piles marking the channel, while none were present on the rocks along the shore. This clearly represents a sporadic settlement, probably derived from infected ships.

J. mar. biol. Ass. U.K. (1959) 38, 439–444
Printed in Great Britain

TWO NEW SPECIES OF POGONOPHORA FROM THE NORTH-EAST ATLANTIC

By Eve C. Southward
From the Plymouth Laboratory

(Text-figs. 1–2)

In May 1958 it was thought that all species of Pogonophora so far discovered in the north-east Atlantic belonged to the genus *Siboglinum* Caullery (Southward & Southward, 1958). Since then I have had the opportunity of examining further collections from deep water off the British Isles and Spain. At least two species of multitentaculate pogonophores have been discovered, and re-examination of some material collected in May 1958 shows that one of the species was also present in these collections. Although considerable material belonging to other species remains to be worked up it has been thought worth while to describe these two species first. One is widespread and can be locally abundant, while the other is the largest pogonophore so far found in the Atlantic. Both are the first Atlantic representatives of their respective genera.

All my material was collected during cruises of R.V. 'Sarsia'. I am grateful to Captain C. A. Hoodless and the crew of 'Sarsia' for their continued interest in deep-sea dredging. I am also indebted to my husband and to Dr J. B. Gilpin-Brown who picked out the specimens, and to Dr J. S. Alexandrowicz who very kindly assisted me with the translation of Russian and German.

Oligobrachia ivanovi sp.nov.

Only one specimen of this species has been found. Its tube is black and completely opaque, and was 25 cm long before being broken to remove the animal. The diameter of the tube varies along its length from 0·5 to 0·9 mm. The wall is stiff and is made of several layers of brown material with a transparent lining layer; its outer surface has narrow raised rings at fairly regular intervals (Fig. 1, A). The animal is 95 mm long, and since only 10 mm of the post-annular region of the trunk is present the complete animal must have been considerably longer. Its diameter is about 0·5 mm and its colour is greenish brown with brown tentacles and red blood. There are seven tentacles, coiled together, which are 12 mm long. Each has a double row of pinnules along the inner side (the length of the pinnules being about equal to the diameter of the tentacle) and a band of brown spots on the outer side (Fig. 1, D). The tentacles are joined to the protosoma in a circle just in front of a slight transverse groove (Fig. 1, B). The protosoma is about 0·8 mm long and is separated from the mesosoma by an oblique groove which reaches the anterior point of the bridle on the dorsal side. Patches of white glands are present on the proto- and mesosoma and their positions are shown in Fig. 1, B and C, by heavy stipple. The anterior part of the metasoma or trunk, called the metameric region in other genera, has white epidermal glands arranged in two lateral bands separated by a deep groove ventrally and by two dark brown stripes dorsally. Between the brown stripes lies the dorsal ciliated band which is pinkish white. The white bands are spotted with 3 to 4 rows of clear patches which mark the openings of internal glands. These patches lie in regular oblique lines and show up clearly because they are surrounded by brown rings. This glandular region of the body is 10 mm long. The second region of the metasoma lacks epidermal glands but bears numerous small papillae arranged in two irregular rows along the ventral side for about 50 mm. The rows of small papillae are followed by large papillae, arranged in 3 to 4 rows, extending for 10 mm, while the last 5 mm of the pre-annular region is devoid of all but a few small papillae. Both large and small papillae are armed with

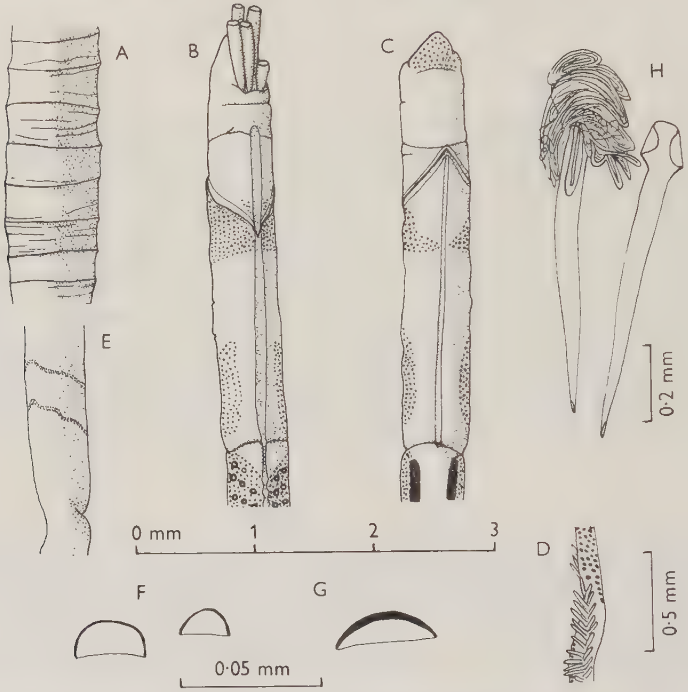


Fig. 1. *Oligobrachia ivanovi*: A, part of tube (surface view); B, anterior end, ventral view; C, anterior end, dorsal view; D, part of tentacle; E, girdle region; F, G, adhesive plates from smaller and larger papillae of trunk; H, spermatophores, filament removed from one.

half-moon-shaped adhesive plates (Fig. 1, G), but the plates are difficult to see because they are almost colourless. There are two girdles (annuli), which both encircle the body completely, and each is made up of 2 to 3 rows of platelets. The last 10 mm of the body has no papillae, adhesive plates or epidermal glands and is broken off abruptly. The pre-annular part of the trunk contains spermatophores which are 0·85 mm long and spindle-shaped. When the filament is removed the filamentar end of the spermatophore is seen to be flattened and drawn out into two flaps, which are usually folded inwards (Fig. 1, H).

The specimen on which this description is based was collected on 28 November 1958 at 48° 26′ N., 10° 8′ W.; depth 730–780 fm.

This new species has been named after Prof. A. V. Ivanov of Leningrad, who has already described 17 species of Pogonophora from the Pacific and Arctic Oceans, including the only other species of *Oligobrachia.*

O. ivanovi differs from *O. dogieli* Ivanov (1957) in having adhesive plates on the trunk papillae, and in this respect it resembles *Birsteinia vitjasi* Ivanov (1952) and confirms Ivanov's thesis that the two genera are closely related. The spermatophores of *Oligobrachia dogieli* and *O. ivanovi* both have wing-like flaps at the filamentar end while there are no such flaps in *Birsteinia* or any other pogonophore yet described. Other characters in common between the two species of *Oligobrachia* are the curious patches or spots on the tentacles, and the comparatively long pinnules. The tentacles in *Birsteinia* have very short pinnules and, apparently, no spots or glands of any sort (Ivanov, 1952).

Polybrachia capillaris sp.nov.

The tubes are dark chestnut brown and look very much like hairs; the specific name *capillaris* refers to this likeness. The tube wall is stiff and, in the anterior part at least, is made up of short overlapping segments (Fig. 2, A). Occasionally the colourless lining layer extends a few mm beyond the first segment. The first few segments are pale brown and unringed; the later segments and the unsegmented part of the tube are marked with regular light and dark brown rings (Fig. 2, A), or half rings. The posterior end of the tube is yellow; the wall is thinner, but still ringed, and is occasionally surrounded by short segments of a darker outer layer. The diameter is constant along any individual tube and may be from 0·12 to 0·17 mm in different individuals, but is usually between 0·13 and 0·14 mm.

It is difficult to assess the length of the complete tube or animal, since all the specimens seem to be broken, but the longest fragment of tube is 12 cm long. The animal itself may possibly reach 55 mm. A specimen (from the Spanish material) that seems to be nearly complete has been chosen as the type and its measurements are given below, followed by the range, in parentheses, found in other specimens: diameter 0·12 mm (0·10–0·12); total length 30·6 mm;

length of pre-annular part 10 mm (5–15); length of post-annular part 20·6 mm (up to 39); length of proto- and mesosoma together 0·75 mm (0·5–1·0); length of coiled tentacles 1·5 mm (0·5–2·2).

The pre-annular part of the body grows longer as the gonads and gametes begin to develop. It is less than 7 mm long in immature specimens; 7–10 mm long in females and 10–15 mm long in males.

There are from two to four tentacles but two is the most common number (11 specimens have 2, 3 specimens have 3, 2 specimens have 4) and the type

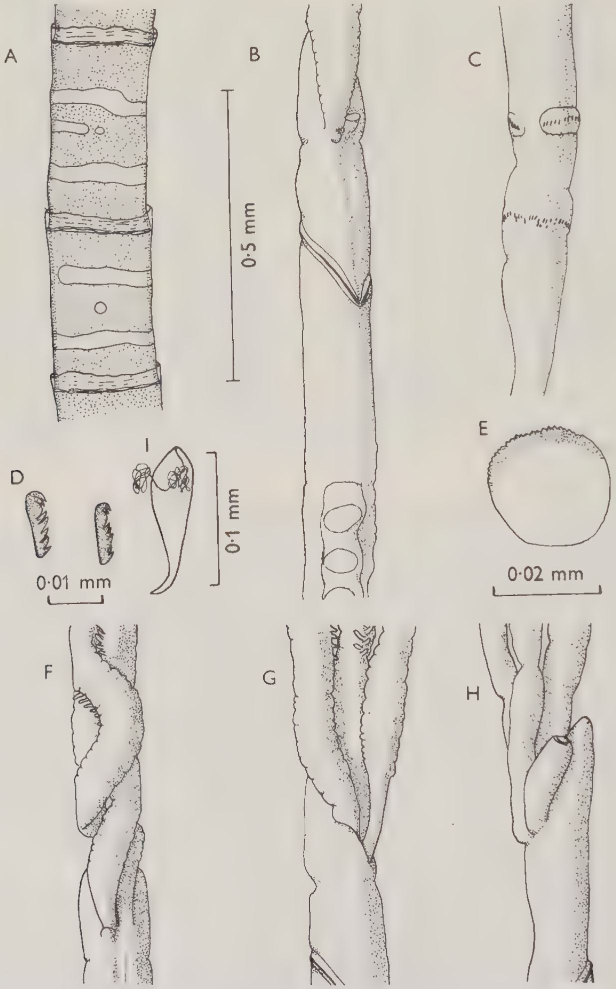


Fig. 2. *Polybrachia capillaris*: A, part of tube; B, anterior end of type specimen, ventral view; C, girdle region; D, girdle platelets (uncini), side view; E, adhesive plate; F, G, H, anterior ends of specimens with 2, 3 and 4 tentacles; I, spermatophore.

specimen has two; they are coiled together into a tight spiral inside the tube and each one has a double row of pinnules (Fig. 2, F, G). Specimens with two tentacles often have a small swelling behind the base of one of them, which may be the first sign of the development of a third. The protosoma is very short (about one-quarter to one-fifth the length of the mesosoma) and is separated from the mesosoma by a shallow groove on the ventral side only (Fig. 2, B). The bridle lies on a broad ridge and is not complete on the dorsal side, but on the ventral side the two halves are sometimes separate (Fig. 2, B) and sometimes joined. There is a wide groove along the ventral side between the base of the tentacles and the bridle.

The septum between the mesosoma and metasoma can be seen through the body wall, but there is no external groove. The metameric region is short and has only 6–13 pairs of glandular papillae (7 in the type specimen). The first 4 or 5 of these papillae are devoid of adhesive plates, but all the other papillae on the metasoma bear small round plates (Fig. 2, E). Behind the paired papillae is a long region bearing occasional isolated papillae and ending in a close row of up to 15 large papillae just before the girdles. The two girdles are made up of single or semi-double rows of small toothed platelets (Fig. 2, C, D). The post-annular region bears transverse rows of two or three papillae at intervals of about 1 mm.

Mature males contain flat, leaf-like spermatophores, with very fine filaments (Fig. 2, I).

Three species of *Polybrachia* are already known. They are: *P. gorbunovi* (Ivanov, 1949), *P. annulata* Ivanov (1952) and *P. barbata* Ivanov (1952). *P. capillaris* has the following characters in common with other species of *Polybrachia*: part of the tube is built up of short overlapping segments; the tentacles bear pinnules; both paired and unpaired trunk papillae bear adhesive plates; the post-annular papillae are arranged in transverse rows, and the spermatophores are of similar shape to those so far described for *Polybrachia*. There are some differences which suggest that the new species might belong to a separate, new, genus: the number of tentacles is small compared with 18–73 in other species; there are no circular grooves on the proto- and mesosoma, apart from the incomplete one separating the two segments; the new species is only about half the size of the smallest species previously known. For the present it seems best not to propose a new generic name for a species obviously closely related to *Polybrachia*.

P. capillaris has been collected at six stations, which are listed below:

Date	Position	Depth (fm.)	No. of specimens
16. v. 58	47° 56′ N., 7° 57′ W.	340–350	1 empty tube
16. v. 58	47° 50′ N., 7° 57′ W.	300–450	3 (and 1 empty)
6. viii. 58	43° 43′ N., 4° 02′ W.	680–970	12 (and many empty)
28. xi. 58	48° 26′ N., 10° 08′ W.	730–780	2 (and 2 empty)
30. xi. 58	48° 39′ N., 9° 50′ W.	750–850	2
30. xi. 58	48° 40′ N., 9° 48′ W.	600–680	1

Some of these specimens were previously recorded as empty tubes of *Siboglinum inerme*[1] (Southward & Southward, 1958), but the description of *S. inerme* was based entirely on genuine specimens.

Type specimens of *Oligobrachia ivanovi* and *Polybrachia capillaris* have been sent to the British Museum (Natural History).

SUMMARY

Two new species of Pogonophora are described. *Oligobrachia ivanovi* is recorded from a depth of 730–780 fm. off the mouth of the English Channel; *Polybrachia capillaris* is recorded from depths of 340–970 fm. at various stations off the mouth of the English Channel and one station off the north coast of Spain.

REFERENCES

IVANOV, A. V., 1949. A new representative of the class Pogonophora. *Zool. Zh. SSSR*, Vol. 28, pp. 78–84. (Russian.)

—— 1952. New Pogonophora from far eastern seas. *Zool. Zh. SSSR*, Vol. 31, pp. 372–91. (Russian.)

—— 1957. Neue Pogonophora aus dem nordwestlichen Teil des Stillen Ozeans. *Zool. Jb.*, Abt. 1, Bd. 85, pp. 430–500.

SOUTHWARD, E. C. & SOUTHWARD, A. J., 1958. On some Pogonophora from the north-east Atlantic, including two new species. *J. mar. biol. Ass. U.K.*, Vol. 37, 627–32.

[1] The name *S. inermis* was used in the original description, it should of course be *S. inerme*.

J. mar. biol. Ass. U.K. (1959) 38, 445–453
Printed in Great Britain

OBSERVATIONS ON HERRING SPAWNING AND LARVAL DISTRIBUTION IN THE FIRTH OF CLYDE IN 1958

By B. B. Parrish, A. Saville, R. E. Craig,
I. G. Baxter and R. Priestley

The Marine Laboratory, Aberdeen

(With Plate I and Text-fig. 1)

Apart from the extensive egg surveys carried out by Norwegian workers (Runnstrom, 1941) most of the investigations on the spawning of the Atlantic Herring have depended on studies of the distribution of the spawning fish, on captures of newly hatched larvae, and on records of the occurrence of herring eggs in the stomachs of predatory fish species (principally haddock). With the exception of recent observations by Bolster and Bridger (1957), attempts to sample egg concentrations quantitatively in the North Sea and neighbouring areas have usually proved abortive. In consequence little is known of the distribution and density of eggs on the spawning grounds, their percentage fertilization, mortality during the egg stage, hatching rate, and the relationship between the distribution of eggs and the nature of the sea-bed.

To study these items, and also the subsequent production, dispersal, and mortality of larvae, and the relationship of these to subsequent year-class strength, it was decided to concentrate effort on the spring spawning in the Firth of Clyde (see Fig. 1). This area had been studied much earlier by Cossor Ewart (1884), and it was considered very suitable for intensive study for the following reasons: (i) spawning occurs in a relatively small, well defined, area of shallow depth (13–24 m); (ii) the spawning season is short, extending over a period of about one month; (iii) the main spawning ground is the scene of a small commercial fishery from which detailed information on the distribution and composition of the spawning shoals can be readily obtained; (iv) the dispersal of the larvae can be followed more closely than in the much more extensive regions of the northern North Sea; (v) the region is one in which accurate fixing of position by Decca and land bearings is possible.

The general features of the biology of the herring in the Clyde have been described by Marshall, Nicholls and Orr (1937, 1939) and Wood (1951, 1958, 1959). Spawning takes place in late February and March in two localities in the outer reaches of the firth, a major one on Ballantrae Bank off the Ayrshire coast of the Scottish mainland, and a smaller one off the south-west corner of the island of Arran. These are shown in Fig. 1. Hatching occurs throughout

March and early April, followed by the dispersal of the larvae from the vicinity of the spawning grounds. The main nursery areas for young herring are located in the upper reaches of the firth where they remain until the onset of first maturity in their third or fourth years of life. After spawning the adults move out of the firth. Only a small proportion of these adults return to the Clyde spawning grounds in subsequent years.

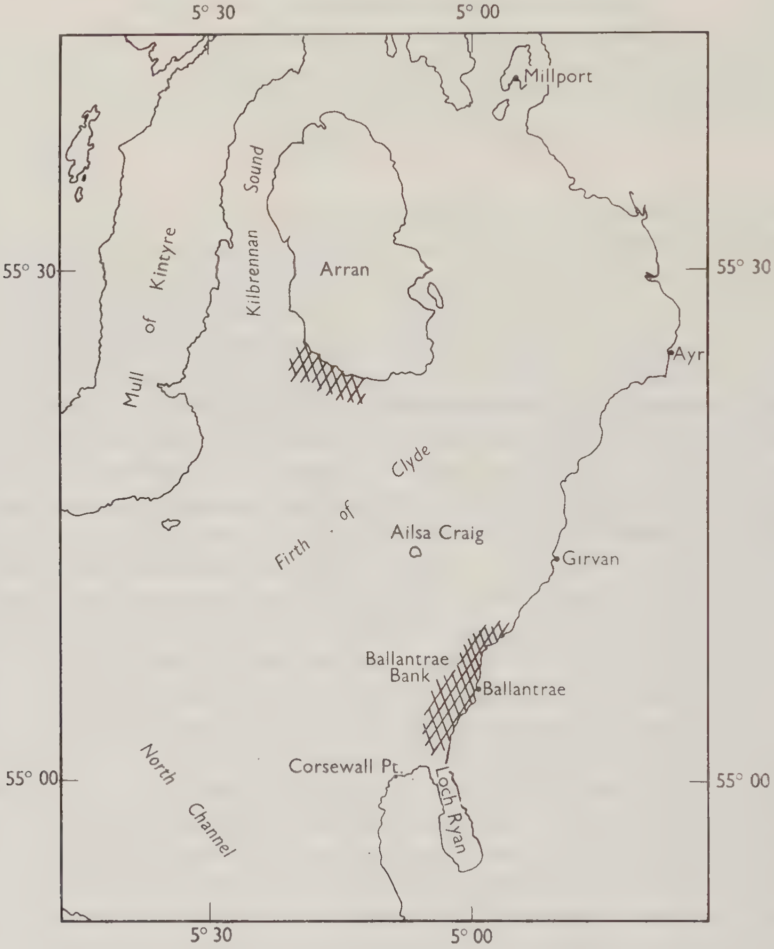

5° 30
5° 00
Millport
Sound
Kilbrennan
Arran
Mull of Kintyre
55° 30
55° 30
Ayr
Clyde
Firth of
Ailsa Craig
Girvan
Ballantrae Bank
Ballantrae
North Channel
Corsewall Pt.
Loch Ryan
55° 00
55° 00
5° 30
5° 00

Fig. 1. Chart of outer reaches of Firth of Clyde showing main spawning areas (hatched).

SAMPLING OF EGG CONCENTRATIONS

In 1957 and 1958, surveys of egg concentrations have been restricted to the larger of the two spawning grounds, Ballantrae Bank. Preliminary work in February and March 1957, and again in greater detail during the same period in 1958, consisted of an intensive survey by a continuous series of dredge hauls over the full extent of the bank. This provided a detailed picture of the nature of the sea-bed and the presence or absence of eggs. An egg patch was located in both 1957 and 1958, but only in 1958 was it possible to investigate the extent of the patch and the distribution and density of the eggs.

The procedure in 1958, after the egg patch had been located by dredge on 4 March, was to mark the position accurately by buoys and with Decca fixes, and then to sample the full extent of the patch with a small grab (mouth aperture 20 × 20 cm), designed for sampling in conjunction with the laboratory's underwater T.V. unit (shown in Pl. I A). By this means, and using the Decca track plotter, it was possible to delimit accurately the boundaries of the patch, to determine the distribution of the eggs and to estimate their total abundance and density. A series of underwater colour transparencies was also taken over the patch using the laboratory's Mk V camera equipped with electronic flash. This proved very satisfactory as a survey instrument for this type of work, and black and white prints, taken from the colour transparencies, are shown in Pl. I B.

The most important results obtained from the dredge, grab and photographic surveys are as follows.

(1) The eggs sampled in both years were located in an area of small stones and gravel. No eggs were found in areas of large stones, boulders or rock.

(2) The egg patch surveyed in detail in 1958 was sharply delimited, the boundaries coinciding with a change in the substratum from gravel and small stones to large stones and rock. The patch was approximately square, measuring 320 × 320 m.

(3) Eggs were distributed in an almost continuous carpet, ranging from 1–2 eggs thick at the boundaries to 4–8 eggs thick over the greater part of the patch.

(4) Detailed examination of eggs taken from different parts of the patch indicated that no hatching had taken place up to the time of locating it. The stage of development varied markedly throughout the egg layer. The eggs in the surface layer were in an advanced stage of development near to hatching, while those in the lower layers were in earlier stages of development. These observations suggest that the eggs were all deposited at approximately the same time, and that the development of the eggs in the lower layers was retarded, due possibly to reduced oxygen supply.

(5) Very few unfertilized or dead eggs were observed in the samples taken from the patch, and there was no significant increase in the thickest layers of eggs. In the samples of eggs examined, the proportion of unfertilized or dead eggs did not exceed 1 %.

(6) No invertebrate fauna was observed over the whole extent of the egg patch, although dense concentrations of ophiuroids were sampled on a similar substratum in the vicinity of the patch and on other parts of the bank.

Abundance of eggs and numbers of spawners

From a knowledge of the size of the egg patch, the distribution of eggs within it, the thickness of the egg layers, and the average egg diameter, it is possible to estimate the total number of eggs in the spawning patch. The number of spawning females and the total number of spawners producing the patch can then be calculated from data on the average fecundity and sex ratio.

Estimates of these quantities are given in Table 1. These estimates are based on there being a continuous carpet of eggs over the patch, and they are given for upper (5) and lower (2) levels of egg layer thickness, within which the actual average egg layer thickness certainly lay. (The average thickness of 50 samples of eggs taken over the patch was 4.) The fecundity value used was for fish of 26–27 cm, which corresponded to the mode of the length composition of the catches taken by the commercial fishery on the bank, and the 50:50 sex ratio used does not differ significantly from that observed in the commercial samples.

TABLE 1. ESTIMATES OF EGGS IN PATCH AND NUMBER OF SPAWNING FISH

	Size of egg patch (m^2)	Diameter of egg (mm)	Estimated total no. of eggs	Average fecundity	Sex ratio	Estimated no. of spawners		
						♀	♂	Total
Egg layer thickness = 2	$1{\cdot}01 \times 10^5$	1·4	$1{\cdot}03 \times 10^{11}$	$3{\cdot}0 \times 10^4$	50:50	$3{\cdot}4 \times 10^6$	$3{\cdot}4 \times 10^6$	$6{\cdot}8 \times 10^6$
Egg layer thickness = 5	$1{\cdot}01 \times 10^5$	1·4	$2{\cdot}58 \times 10^{11}$	$3{\cdot}0 \times 10^4$	50:50	$8{\cdot}6 \times 10^6$	$8{\cdot}6 \times 10^6$	$17{\cdot}2 \times 10^6$

These calculations indicate that the total number of herring of both sexes contributing to the spawning patch lay between 7 million and 17 million fish. The estimates of average egg layer thickness obtained during the survey suggest that the actual number was nearer the upper of these two limits, and that 10 million fish represents a reasonable conservative estimate.

That this spawning patch did not constitute the total spawning on Ballantrae Bank during the whole of the spawning season in 1958 is evident from other information. Ripe herring were caught by the commercial fishery on the bank after the time that the egg patch had been located; larval records obtained subsequent to the location of the egg patch indicated that substantial spawning took place after this time. Also running fish and a few spents were caught in the fishery on the bank at a time before the formation of the sampled egg patch. The general course of the fishery and the subsequent larval data indicate that the sampled patch was formed near the beginning of the spawning season, and that substantial spawning took place on the bank subsequently. From these data it is estimated that the total number of spawners on the bank during the spawning season was 4–5 times greater than the number contributing to the sampled egg patch. This gives rough limits for the total spawning stock size of between 30 million and 85 million fish.

Rate of exploitation

A comparison of these estimates of the size of the spawning stock with the catches taken by the commercial fishery on the bank, during the spawning season, provides information on the rate of exploitation. This fishery is pursued with anchored gill nets during February and March. The anchored nets are shot in fleets of 10 across the bank; they are set to fish throughout 24 h, and are usually hauled in the morning. The positions of the nets are changed from time to time according to the regions on the bank at which the heaviest catches are being taken.

The statistics of this fishery in 1958, from its commencement at the beginning of February to its termination in late March, are given in Table 2.

TABLE 2

Week	Total no. net-days	Total catch (crans)
3–8 February	120	39·5
10–15 February	750	185·0
17–22 February	750	207·75
24 February–1 March	750	65·5
3–8 March	750	66·5
10–15 March	570	77·5
17–22 March	450	16·25
Total	4140	658·0

Estimates of the proportion of the total spawning stock taken by the fishery can be made from these catch data and the estimates of spawning stock size, both for the total spawning season and for the spawners contributing to the sampled egg patch. The number of herring per cran in this fishery averaged approximately 1000, which gives a total catch during the season of about 658000 herring. This constitutes a fraction of between

$$\frac{0{\cdot}658\times10^6}{85{\cdot}0\times10^6}=0{\cdot}8\,\% \quad \text{and} \quad \frac{0{\cdot}685\times10^6}{30{\cdot}0\times10^6}=2{\cdot}2\,\%$$

of the total number of spawners on the bank.

Similarly, estimates can be made of the herring contributing to the sampled spawning patch which were taken by the fishery. From the stage of development of the eggs in the patch it is estimated that they were fertilized 12–13 days previously. This fixes the spawning date as on or about 22 February. The catch by the fishery up to this date amounted to 432 crans, and on the assumption that the herring available to the fishery up to this time all contributed to the sampled spawning patch, the estimate of the proportion taken by the fishery up to this time lies between

$$\frac{0{\cdot}432\times10^6}{17{\cdot}2\times10^6}=2{\cdot}5\,\% \quad \text{and} \quad \frac{0{\cdot}432\times10^6}{6{\cdot}8\times10^6}=6{\cdot}4\,\%$$

These estimates are in fact over-estimates, since it is known that in the early part of the season some spawning took place over parts of the bank other than the sampled patch. It is evident therefore that the seasonal fishing mortality rate of this spawning stock in 1958 was probably between 1 and 3%.

LARVAL ABUNDANCE AND DISTRIBUTION

The larval surveys carried out in 1958 subsequent to the egg surveys were planned with the two primary objectives of assessing the numbers of eggs spawned over the season, and of tracing the subsequent dispersal of the larvae produced from them. The first of these aims demanded a close 'spawning' grid of stations covering the limited area on Ballantrae Bank within which spawning was known to take place, to be worked and repeated at frequent intervals; the second required a grid of more widely spaced stations over the Clyde estuary. The attempt to fulfil both of these requirements inevitably resulted in sampling falling rather short of the ideal in both cases.

The 'spawning' grid of sixteen stations was completed fifteen times between 21 February and 21 April. Sampling was by means of oblique hauls from bottom to surface with a one metre net of bolting silk with 60 threads to the inch.

Recently hatched larvae were found from 3 March to 9 April. Hatching was greatest, however, between 10 and 14 March, with a distinct but very secondary mode around 7 April. Only negligible production of larvae occurred in the intervening period.

An indispensable preliminary to assessing the numbers of larvae produced was to estimate the rate at which larvae were removed from the bank. It was possible to estimate this from the rate at which the numbers on the bank fell off between 18 and 31 March, and again after 7 April, during which periods no, or only negligible, new production of larvae was taking place. In both of these periods the rate of loss from the bank was estimated to be about 75% per day. This loss is of course compounded of two factors, dispersion and mortality, but for this purpose it is not necessary to distinguish between them. This rate has been taken as applicable throughout the entire hatching period. Other assumptions made in estimating the number of larvae hatched were that the distribution was linear in space between stations, and in time between surveys. The former is not likely to lead to serious error in this case, where the stations were less than 1 mile apart. The latter is less reliable where, as happened on one occasion, surveys were separated by a period of 6 days, with a marked fall in larval numbers in the intervening period.

On this basis the number of larvae hatched on Ballantrae Bank during the 1958 spawning season was estimated at $5{\cdot}4 \times 10^{11}$. This would be the progeny of about $21\frac{1}{2}$ million spawning females if one assumes that all the eggs spawned subsequently hatched. (It has been shown in an earlier section that the proportion of dead or unfertilized eggs in the sampled patch was low, and no large concentrations of predators were encountered. It is likely, therefore, that mortality in the egg stage was low and that this assumption is reasonable.) This estimate is in broad agreement with that deduced from the estimation of the egg patch. It would suggest, as shown earlier, that there were perhaps four or five egg patches present on the bank during the course of the spawning season.

The larger grids, designed to follow the fate of the larvae subsequent to hatching, were completed eight times between 10 March and 22 April. The first two surveys were carried out on 10 and 12 March. On both of these larvae were sharply restricted to a narrow belt in the vicinity of Ballantrae Bank, and were evidently drifting southwards towards Loch Ryan and round Corsewall Point. Unfortunately there was then a gap of six days before the wide grid was resampled. During this period two marked changes had occurred. First, the numbers of larvae present in the survey area had fallen to less than 1% of their previous abundance; secondly, the sharp western boundary to the distribution of larvae had broken down and the few that remained were widely dispersed westwards to the limit of the survey area. That the marked reduction in abundance of these larvae was the result of drift westwards beyond the limits of the area surveyed, and probably out of the Clyde altogether, is supported by returns from drift bottles released over Ballantrae Bank during this period. The percentage return from these releases was very low, the few recovered being returned from the shores of Kintyre. The most plausible explanation of the low returns is that the remaining bottles were lost to the Atlantic.

The remnants of this group of larvae, which represented the preponderance of the season's production, only suffered a moderate mortality thereafter. By 3 April they were split into a smaller group in Kilbrennan Sound and a larger body off Corsewall Point and the mouth of Loch Ryan. These two groups retained their identity thereafter, the Kilbrennan Sound one being later found to the east of Arran, while that to the south showed a pulse-like movement, first moving south into the Irish Sea and then north again into the Clyde. This was also true of the larvae hatched during the second peak in early April, which showed distinct evidence of the same oscillation of the main body between the Clyde and the Irish Sea, with smaller bodies of larvae subsequently drifting northwards. These movements also are in substantial agreement with returns from later releases of drift bottles, which gave evidence of a southwards drift into the Irish Sea and subsequent return to the Clyde, with a predominantly northerly drift to the Ayrshire coast in the latter half of April.

Surveys of the sea lochs in the upper reaches of the firth were undertaken in late April and early May from M.V. 'Calanus', by kind permission of the

Director of the Scottish Marine Biological Association Laboratory at Millport. They showed no evidence of larvae in these regions, in striking contrast to results in 1957, when large numbers of larvae were captured in these lochs at that time. The difference would seem to be due primarily to the different water movements in the two years. In 1958 there was a large loss of larvae from the Clyde due to predominantly westerly and southerly transport, while in 1957 the northerly drift tended to retain the larvae within the Clyde estuary. These water movements may be largely wind determined. In 1957 winds were predominantly southerly during the last three weeks of March, whilst in 1958 during this period the prevailing winds were from the east.

SUMMARY

In the period February–April 1958 an intensive survey was carried out on the herring spawning grounds of Ballantrae Bank, in the outer reaches of the Firth of Clyde, and over the regions of subsequent larval dispersal.

During a detailed dredge and grab survey of the bank an egg patch, measuring approximately 320 × 320 m was located. This was confined to a gravel and small stone substratum. The eggs were distributed as an almost continuous carpet, with the layers of eggs ranging from two at the boundaries of the patch to eight in its densest parts. Very few dead or unfertilized eggs were observed, but the stage of development varied through the egg layer.

Estimates of the number of eggs in the patch lay between $1 \cdot 03$ and $2 \cdot 58 \times 10^{11}$ and of the number of spawners giving rise to them between 7 million and 17 million. These gave estimates for the total number of herring spawning on the bank during the season of between 30 million and 85 million.

Using these estimates of total spawning stock size, the seasonal fishing mortality rate was estimated to lie between 1 and 3%.

Estimates of total spawning stock size obtained from data collected on subsequent quantitative larval surveys over the bank were in broad agreement with those derived from the egg survey. The dispersal of larvae from the bank was rapid and widespread, and a large proportion of the larvae were probably carried out of the Clyde estuary. The dispersion differed from that in 1957, when the bulk of the larvae were retained within the Firth of Clyde, and it corresponded with the dispersion of drift bottles, liberated on Ballantrae Bank during the hatching season.

REFERENCES

Bolster, G. C. & Bridger, J. P., 1957. Nature of the spawning area of herring. *Nature, Lond.*, Vol. 179, 638.

Ewart, J. C., 1884. Natural history of the herring. *Rep. Fish. Bd. Scot.*, Vol. 2 (1883), pp. 61–73.

Marshall, S. M., Nicholls, A. G. & Orr, A. P., 1937. Growth and feeding of the larval and post-larval stages of the Clyde herring. *J. mar. biol. Ass. U.K.*, Vol. 22, pp. 245–67.

(*Facing p.* 453)

MARSHALL, S. M., NICHOLLS, A. G. & ORR, A. P., 1939. 'On the growth and feeding of young herring in the Clyde.' *J. mar. biol. Ass. U.K.*, Vol. 23, pp. 427–55.

RUNNSTROM, S., 1941. Quantitative investigations on herring spawning and its yearly fluctuations at the west coast of Norway. *Fiskeridir. Skr. Havundersøk.*, Bd. 6, No. 8, 71 pp.

WOOD, H., 1951. Observations on the value of l_1 in herring investigations. *Rapp. Cons. Explor. Mer*, Vol. 128, Pt 1, pp. 39–46.

—— 1958. Race characters of the herring of the Clyde Estuary. Summary. *Rapp. Cons. Explor. Mer*, Vol. 143, Pt 2, p. 65.

—— 1959. (Paper in press).

EXPLANATION OF PLATE I

A. Grab used in egg survey, with egg sample taken in a single haul.
B. Print of eggs *in situ* taken with underwater camera.

J. mar. biol. Ass. U.K. (1959) 38, 455–460
Printed in Great Britain

NAPHTHAQUINONE PIGMENTS IN *PSAMMECHINUS MILIARIS* (GMELIN)

By M. YOSHIDA

Zoology Department, Bedford College, University of London

(Text-figs. 1–3)

Since MacMunn (1885), the nature and distribution of naphthaquinone pigments in sea urchins have been widely surveyed, and the main findings have been summarized in reviews by Lederer (1952), Fox (1953) and Thomson (1957). Among spinochromes and echinochromes, the most unusual one is the Spinochrome E discovered by Lederer (1952), which is insoluble in ether.

Recently, the author has examined the napthaquinone pigment in the echinoid, *Psammechinus miliaris* (Gmel.), and has found a similar pigment to Spinochrome E together with other common spinochromes.

SPINOCHROME E

Methods

Tests and spines were digested with concentrated HCl and extracted with re-distilled diethyl ether. The spectral characteristics were measured on Unicam SP500. The crude ether phase showed absorption maxima at 269, from 315 to 318 and at 480 mμ with a hump at 360 mμ (Fig. 2*a*). When this was left for a short time at room temperature, needle-like crystals occasionally appeared, which, when dissolved in methanol, showed maxima at 267, between 359 and 360 and at 476 mμ (Fig. 2*b*).

A considerable amount of orange red pigment remained in the acid phase ($\lambda_{\text{max.}}$; 261, 330 and 465 mμ in HCl), which on adding a few drops of pyridine turned purple. Dilution with methanol produced an amorphous purple precipitate, which re-dissolved in methanol on acidification with a drop of concentrated HCl. From this solution, fine needle crystals separated out at room temperature. These, when dissolved in methanol, gave $\lambda_{\text{max.}}$ at 267, 358 and 476 mμ and the absorption curve (Fig. 1*b*) was exactly the same as that from the ether phase.

A more effective method of crystallization was as follows. Tests and spines were digested with a small amount of concentrated HCl and the solution was diluted at once with methanol, which gave a solution with the absorption spectrum shown in Fig. 1*d*. On adding a small amount of pyridine, the purple pigment precipitated in quantity. This was washed several times with methanol, dried and re-dissolved in acidified methanol or acetone. Crystals separated out at room temperature, which after washing with distilled water and drying, were re-crystallized from acidified methanol.

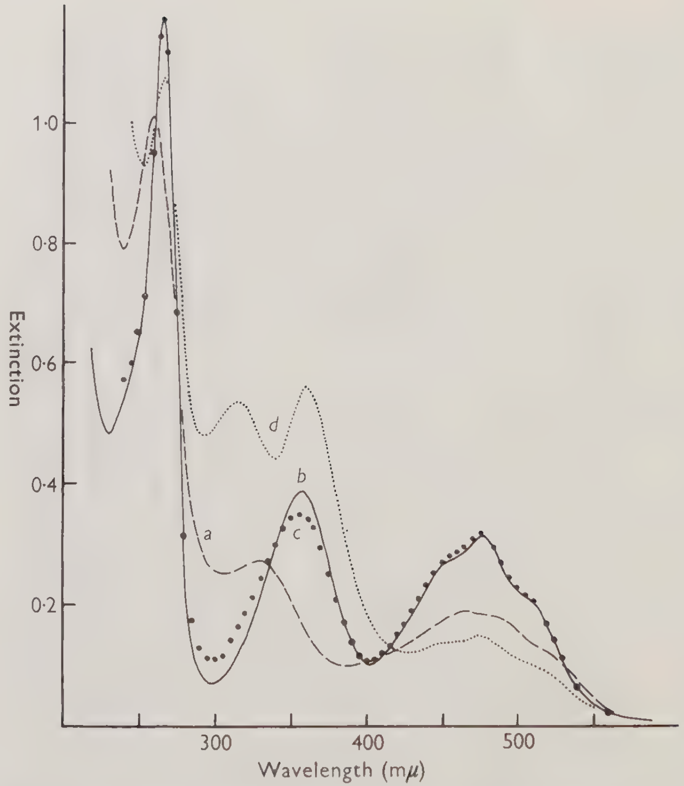


Fig. 1. Absorption spectra of pigment extracted from *Psammechinus miliaris*. HCl phase. *a* (broken line), crude extract in HCl; *b* (continuous line), crystallized pigment in methanol; *c* (dots), samples of Spinochrome E, furnished by Dr Lederer; *d* (dotted line), crude extract made with HCl methanol.

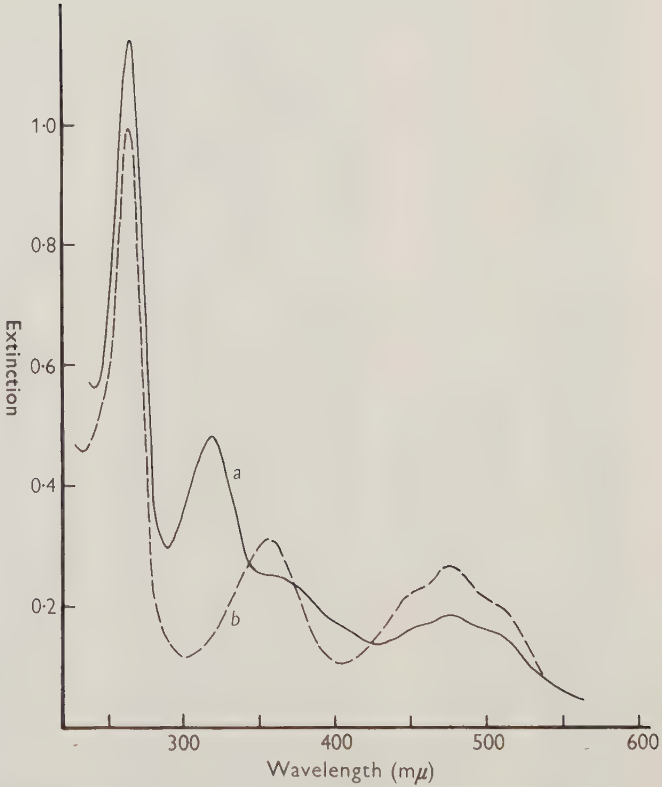


Fig. 2. Absorption spectra of pigment extracted from *Psammechinus miliaris*. Ether phase. *a* (continuous line), crude extract in ether, *b* (broken line), Spinochrome E in methanol.

Properties

The properties of this pigment resemble very closely those of Spinochrome E. On adding sodium hydrosulphite, the solution turns colourless but the colour soon comes back on contact with air. The pigment forms a slightly reddish yellow sodium salt when sodium bicarbonate is added to a methanolic solution. On acidifying with HCl, the spectral character of the original solution is restored.

The spectral absorption of a sample of Spinochrome E, kindly sent to me by Dr E. Lederer, was found to be almost identical with that of the above pigment, showing only a slight difference in the relative extinction at the vicinity of the middle peak (Fig. 1*c*).

The two pigments were compared by paper-chromatography, by running them in parallel with 2N-HCl/methanol (1:5) as developer. The R_F was found to be 0·39–0·41 for Lederer's and 0·38–0·41 for my samples of pigment. None of the pigments moved from the origin when the chromatogram was developed by butanol–water–1% formic acid. This solvent system, devised by Dr Barbier, was recommended to me privately by Dr E. Lederer.

The crystals from *Psammechinus* do not melt below 320° C, the highest temperature tested, and sublime at 280° C (according to Lederer (1952) his pigment did not melt below 350° C).

Both pigments were found to be insoluble in ether, carbon disulphide, chloroform and benzene, but very soluble in acetone, methanol and ethanol.

Thus the two pigments are closely similar in solubility, absorption spectra, melting-points and in their behaviour on paper chromatograms. The pigment found in *Psammechinus*, therefore, appears to be Spinochrome E.

An elementary analysis of the pigment after two successive re-crystallizations showed $C = 41{\cdot}66\%$, $H = 3{\cdot}82\%$ and O (by difference) $= 54{\cdot}52\%$. The empirical formula which corresponds most closely to this is therefore $C_{10}H_{11}O_{10}$.

On addition of weak sodium hydroxide, the optical density in the visible relative to that in the ultraviolet range decreases, with an enhancement of the hump at 455 mμ, as well as a shift of the first peak to 270 mμ. If 0·5 c.c. of 20% NaOH is added to 5 c.c. of methanolic solution, the second peak (at 358 mμ) and the third (476 mμ) disappear completely, leaving a peak at 278 mμ and a hump at 300 mμ.

It is noteworthy that the pigment from *Psammechinus* shows a change during extraction. Thus after it has crystallized out of a crude extract made with either ether or HCl, it will not re-dissolve in either solvent.

Distribution

The pigment was found not only in tests and spines but also in the coelomic fluid. It was not detectable, however, in extracts of guts and gonads.

Discussion

In general, though the properties described above are well known and characteristic of hydroxynaphthaquinones, certain reservations must be made. First, the pigment is remarkably unstable. Although the crystallized pigment is so stable that it gives exactly the same absorption curve after several months storage, crude extracts go black overnight even when acid. Again, when a drop of 2% hydrogen peroxide is added to 10 c.c. of pigment solution, the colour disappears almost instantaneously. This may explain the fact that, if a crude HCl solution is shaken with ordinary ether, which usually contains small quantities of peroxide, the red colour fades within 30 min. Therefore, only pure re-distilled ether should be used during the course of extraction. Again, the absorption spectrum of the pigment eluted from alumina columns by acid methanol is altered greatly.

Secondly, there is the question of the elementary composition, which does not correspond closely to that expected for a hydroxynaphthaquinone, the value for O being too high.

Nevertheless, the three absorption peaks are characteristic of a hydroxynaphthaquinone (Spruit, 1949), as is the behaviour of the pigment towards sodium hydrosulphite, sodium bicarbonate and sodium hydroxide. Thus the shift of the peak towards the longer wavelength in alkali (see p. 457) is more or less in harmony with Spruit's ideas. The infra-red spectrum reveals nothing unusual for a hydroxynaphthaquinone.

The change in solubility occurring during the course of extraction is noteworthy and may indicate that the naturally occurring pigment differs somewhat from that which is crystallized from extracts. A similar change occurs when pyridine is used as described on p. 455, for the crystals which are deposited are insoluble in HCl, although the original pigment was extracted with it. Spinochrome E cannot therefore be accepted unreservedly as a naturally occurring pigment.

However, the spectral absorption of the pigment is consistent. Crude extracts with both HCl-methanol (Fig. 1 *d*) and ether (Fig. 2 *a*) show either a small peak or a hump at the vicinity of 360 mμ in addition to a peak at 320 mμ; the latter is clearly due to other spinochromes (see p. 459). Thus the peaks in the crystallized pigment at 267, 358 and 476 mμ correspond to those at 268, 360 and 478 mμ in the crude extract.

OTHER PIGMENTS

Other pigments in the ether phase were also studied by the following means. Tests and spines were digested by 2N-HCl and extracted with re-distilled ether. The ethereal solution was washed with distilled water and dried over anhydrous Na_2CO_3. The concentrated pigment solution was chromatographed on $CaCO_3$, which was activated for 3 h at 180° C before use.

By developing with ether, the pigment can be separated into three fractions on the column: (1) a red pigment which is only weakly adsorbed, (2) a blue (sometimes green) zone, which descends slowly, and (3) a violet top zone. The second and the third zones were separated by dissecting the column, dissolving each part in 2N-HCl and taking up the pigment liberated into diethyl ether.

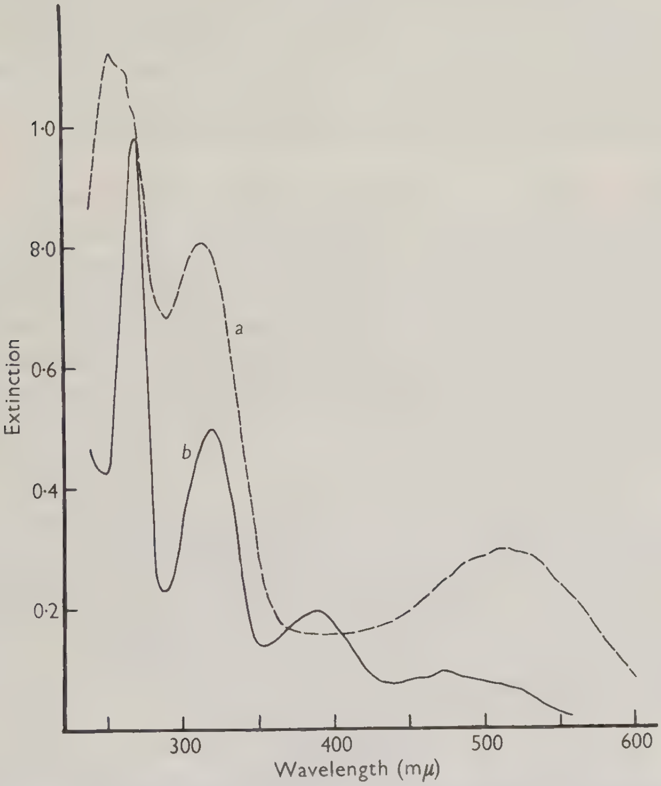


Fig. 3. Absorption spectra of pigment extracted from *Psammechinus miliaris*. Ether phase. *a* (broken line), Spinochrome A in ether; *b* (continuous line), Spinochrome B in ether.

The absorption maxima of the three eluates were as follows and the curves are shown in Fig. 3:

Zone	Solvent	λmax. (mμ)			
Red	Ether	269	320	390	470
Blue or green	Ether (Fig. 3*b*)	272	320/2	388	470
	Methanol	270	318	—	480
Violet	Ether (Fig. 3*a*)	255	315	—	510
	Chloroform	255	318	—	515 535

Although no further tests were performed, the behaviour of these pigments on the chromatogram, their spectral absorption and their coloration on calcium columns, suggest that the violet and blue-green zones are Spinochromes A and B respectively (Goodwin & Srisukh, 1950; Lederer, 1952).

As mentioned by Millott (1957), however, results on the $CaCO_3$ columns are not always consistent, so that the top zone sometimes gives a brown colour and the second blue-green band is sometimes missing. As well as this, the absorption maxima of the top zone vary, values of 266, 315, 395 and 480 mμ having been recorded. This zone may thus be an artefact.

My thanks are due to the Directors and staff of the Marine Stations at Plymouth, Millport and Bangor, where part of the work was performed. I am deeply indebted to Dr T. W. Goodwin of the Department of Biochemistry at the University of Liverpool who obtained for me the melting-point, the infra-red spectrum and the elementary analysis, and to Dr E. Lederer for the samples of Spinochrome E and many helpful suggestions. I am also indebted to Prof. N. Millott for his encouragement and kind advice throughout the work. The investigation was supported by a grant from the Medical Research Council.

SUMMARY

Naphthaquinone pigments in *Psammechinus miliaris* were studied.

After extracting by HCl-ether, the ether phase showed properties indicating the presence of Spinochromes A and B. A pigment resembling Spinochrome E was found in both ether and HCl phases.

An improved method of crystallizing Spinochrome E and some additional properties of the pigment are described.

REFERENCES

Fox, D. L., 1953. *Animal Biochromes*. Cambridge: Univ. Press.

Goodwin, T. W. & Srisukh, S., 1950. A study of the pigments of the sea-urchins, *Echinus esculentus* L. and *Paracentrotus lividus* Lamarck. *Biochem. J.*, Vol. 47, pp. 69–76.

Lederer, E., 1952. Sur les pigments naphthaquinones des épines et du test des oursins *Paracentrotus lividus* et *Arbacia pustulosa*. *Biochem. Biophys. Acta*, Vol. 9, pp. 92–101.

MacMunn, C. A., 1885. On the chromatology of the blood of some invertebrates. *Quart. J. micr. Sci.*, Vol. 25, pp. 469–90.

Millott, N., 1957. Naphthaquinone pigment in the tropical sea urchin, *Diadema antillarum* Philippi. *Proc. zool. Soc. Lond.*, Vol. 129, pp. 263–72.

Spruit, C. J. P., 1949. Absorption spectra of quinones. I. Naphthoquinones and naphthohydroquinones. *Rec. Trav. chim. Pays-Bas*, T. 68, pp. 309–24.

Thomson, R. H., 1957. *Naturally Occurring Quinones*. London: Butterworths.

J. mar. biol. Ass. U.K. (1959) 38, 461–467
Printed in Great Britain

THE LARVAL DEVELOPMENT OF TWO SPECIES OF GASTROCOTYLID TREMATODE PARASITES FROM THE GILLS OF *TRACHURUS TRACHURUS*

By J. Llewellyn

Department of Zoology and Comparative Physiology, University of Birmingham

(Text-figs. 1–9)

Plymouth scad (horse-mackerel) *Trachurus trachurus* (L.) are known to harbour three species of monogenean trematode gill parasites, of which two belong to the Gastrocotylidae and one to the Microcotylidae. The oncomiracidia (= newly hatched larvae) of these parasites have already been described (Llewellyn, 1957*a*), but nothing is known of the developmental stages intervening between the oncomiracidia and the adults. Bychowsky (1957) has stated that some information is available about the larval development of 13 monogenean families, leaving 15 families, including the Gastrocotylidae, about whose larval development nothing is known. The ontogenetic development of the Gastrocotylidae is of especial interest since the oncomiracidia are bilaterally symmetrical, but the adults invariably show an extreme degree of asymmetry.

In spite of a rigorous search, no larval monogeneans were found on mature *Trachurus* (20–30 cm long) at Plymouth during July and August 1954–8. Even in smaller scad (10–15 cm) examined in the same months in 1957 and 1958, all the parasites were found to be mature and egg-laying.

On 6 May 1959 about 50 specimens of *Trachurus trachurus*, of 9·3 to 12·6 cm length, were landed at Plymouth and sent in ice to Birmingham. Here 19 out of a sample of 20 of these young scad were found to bear an abundance of larval monogeneans, and immediately some new problems presented themselves: (*a*) is there a seasonal rhythm in the reproductive cycle of the monogenean parasites? and (*b*) do these particular monogenean larvae become parasitic only on young scad? It was of course realized that these questions would be best answered by continuous observations over a long period, but since this was impracticable, it was thought that some indications might emerge from comparing the parasite populations from hosts of different ages. Accordingly, through the most helpful co-operation of the staff of the Plymouth Laboratory, some large *Trachurus trachurus* caught off Plymouth on 20 May were measured individually to permit estimates to be made of their ages, then deep-frozen, and later the heads and gills were sent in vacuum flasks to Birmingham.

The gills of all the fishes were searched carefully with a stereomicroscope, and the dead parasites were rinsed in sea water and preserved in 4% formaldehyde. Some specimens were stained in haematoxylin or carmine and mounted in Canada Balsam, and some were sectioned at 6 μ, but most were mounted freely without pressure of a coverglass, in 4% formaldehyde, to permit measurements to be made.

The parasites were identified as *Gastrocotyle trachuri* van Beneden & Hesse (present relatively abundantly) and *Pseudaxine trachuri* Parona & Perugia (present relatively rarely); no microcotylids were found. A combined total of 182 larval and adult *Gastrocotyle* was found to be distributed among 24 host fishes as indicated in Table 1.

TABLE 1

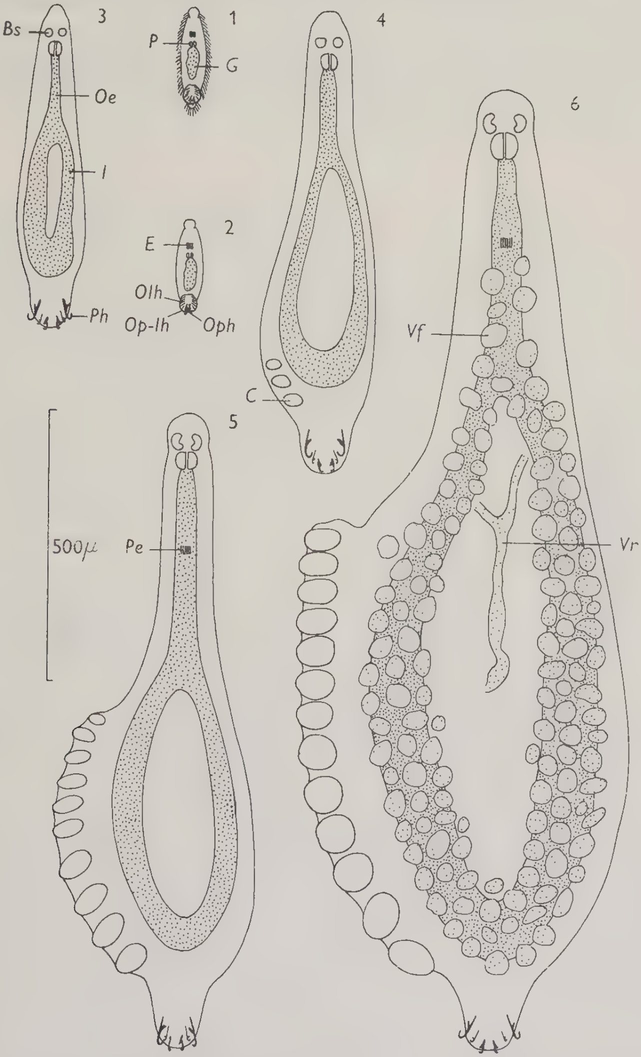
Length of host (cm)	Probable age of host* (yr)	No. of host specimens examined	No. infested	Total no. of parasites	Mean no. of parasites/host	Mean and range of no. of clamps/parasite
Under 12·9	Up to 1	10	9	116	11·6	12·5 (0–21)
13·0–18·9	1–2	3	3	37	12·3	20·4 (9–35)
19·0–23·9	2–3	6	5	25	4·2	21·0 (0–36)
Over 24·0	Over 3	5	1	4	0·8	15·8 (12–17)

* Based on information kindly supplied by the late Dr G. A. Steven (Marine Biological Association of the United Kingdom, Report of the Council for 1957–58, p. 15).

THE LARVAL DEVELOPMENT OF *GASTROCOTYLE TRACHURI*

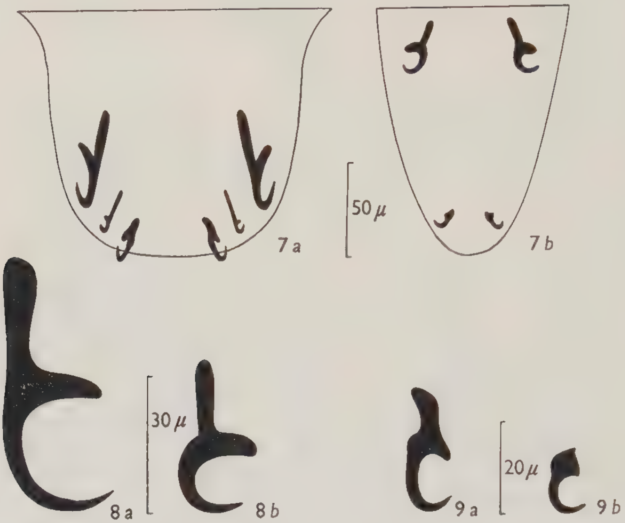
The oncomiracidium, about 0·16–0·20 mm in length (Figs. 1, 2) has been described previously (Llewellyn, 1957*a*), but subsequent observations during the course of abortive attempts to infect adult *Trachurus* with oncomiracidia have shown that the postero-lateral hooks may be as long as 26 μ, and a more accurate expression of their size range is 23 μ (19–26 μ). The posterior hooks are 26 μ (23–28 μ) in length.

The earliest post-oncomiracidial larvae found were bilaterally symmetrical and about 0·50–0·85 mm long (Fig. 3). These larvae have lost the eyes and the 4 pairs of lateral hooks of the oncomiracidium, the place of the lateral hooks being taken, functionally if not topographically, by a pair of relatively large hooks each 54 μ (52–56 μ) long (Figs. 3, 8*a*). These hooks in *Gastrocotyle* obviously correspond to those hooks in *Microcotyle labracis* which I described as 'primordial adult hooks' (Llewellyn, 1957*a*). It is noteworthy that all three pairs of hooks (i.e. the oncomiracidial postero-lateral and posterior hooks, and the newly acquired 'large hooks') present in *Gastrocotyle* at this stage persist without change of size or shape throughout all succeeding larval stages and survive in the definitive adult on the 'anchor-bearing lappet' (Fig. 7*a*) that has been described in many monogeneans. The alimentary canal, which in the oncomiracidium consisted merely of a mouth, pharynx, and simple sacculate intestine, is now provided with a pair of buccal suckers; the gut is differentiated into an oesophagus that bifurcates into two intestinal limbs and these become confluent posteriorly. The walls of the oesophagus and the intestine are lined by scattered pigment cells indicating, by analogy with what is known about similar cells in adult polyopisthocotylineans, that the larvae have already been feeding on blood (Llewellyn, 1954).



Figs. 1–6. The larval development of *Gastrocotyle trachuri*; all diagrams drawn to the same scale. Fig. 1. Newly hatched oncomiracidium. Fig. 2. Oncomiracidium after shedding of ciliated epidermis. Fig. 3. Bilaterally symmetrical post-oncomiracidial larva. Figs. 4–6. Immature stages with 3, 10, and 13 clamps respectively. *Bs*, buccal sucker; *C*, clamp; *E*, eye; *G*, gut; *I*, intestine; *Oe*, oesophagus; *Olh*, *Op-lh*, *Oph*, lateral, postero-lateral, and posterior hooks respectively of oncomiracidium; *P*, pharynx; *Pe*, penis; *Ph*, post-oncomiracidial hook; *Vf*, vitelline follicle; *Vr*, vitelline reservoir.

During the next phase (0·60–1·00 mm) asymmetrical development begins: clamps develop on one side only of the posterior region of the body, which becomes slightly wider to accommodate them (Fig. 4). There is a complete absence of clamp development on the other side of the body. The relationship of these unilaterally distributed clamps in the adult to the gill-ventilating current of the host has been discussed previously (Llewellyn, 1956, 1957*b*). Usually the clamps develop singly in strict posterior–anterior succession, but in one specimen the posteriormost clamp was found to be considerably smaller, and presumably younger, than the one anterior to it.



Figs. 7–9. Comparison of immature forms (13-clamp stage) of *Gastrocotyle trachuri* (7*a*, 8*a*, 9*a*) and *Pseudaxine trachuri* (7*b*, 8*b*, 9*b*). Fig. 7. Anchor-bearing lappet. Fig. 8. Post-oncomiracidial hooks. Fig. 9. Oncomiracidial posterior hooks.

The number of clamps continues to increase as the larva grows bigger, and at the 9 or 10-clamp stage (1·0–1·20 mm) the penis sclerites have appeared (Fig. 5). Soon after (10–12 clamps, 1·20–1·40 mm) vitellaria have developed in association with the intestinal caeca, to be followed at the 13–16-clamp stage (1·50–2·00 mm) by the medianly situated vitelline reservoir (Fig. 6). The formation of the remainder of the egg-capsule-forming system takes place at about the 16-clamp stage (1·80–2·20 mm), and several specimens at this stage of development were found each to contain an egg capsule which, however, appeared to be without embryonic (ovum/oocyte/zygote) contents. In fact, no germarium could be identified in whole mount preparations, and so paraffin sections of 13-, 14-, 15- and 16-clamp larvae were prepared. The specimens, having been preserved in ice before histological treatment, yielded comparatively poor sections, but it was possible to determine that the testes and germarium were still immature. The inference is, then, that the egg-capsule-forming apparatus (the vitellarium and the ootype and its associated glands) becomes functional before the truly germinal portions of the genitalia (the testes and germarium). Egg capsules yielding oncomiracidia have been collected from a specimen of *Gastrocotyle* with 19 clamps. A continued steady increase in the total length of the body is accompanied by a correspondingly steady increase in the number of clamps until eventually a maximum of 35 to 40 clamps is present in parasites of about 2·80–3·20 mm long.

THE LARVAL DEVELOPMENT OF *PSEUDAXINE TRACHURI*

The larval development of *Pseudaxine trachuri* is generally similar to that of *Gastrocotyle trachuri*, but the following differences have been found to occur.

Morphologically the oncomiracidium of *Pseudaxine* may be reliably distinguished from that of *Gastrocotyle* only by the smaller posterior hooks, which are 19 μ (18–20 μ) in *Pseudaxine* and 26 μ (23–28 μ) in *Gastrocotyle*. The difference in size of the postero-lateral hooks, which have been found to be 19 μ (18–20 μ) in *Pseudaxine* and 21 μ (20–26 μ) in *Gastrocotyle*, is likely to be a less reliable distinction, especially since the postero-lateral hooks of *Pseudaxine* are destined to reach a length of 24–26 μ before they eventually disappear.

With the acquisition of the post-oncomiracidial hooks the two species of larvae may be readily distinguished from each other: in *Pseudaxine* these hooks are 32 μ (31–33 μ) long whereas those of *Gastrocotyle* are 54 μ (52–56 μ) long. The shapes of these hooks are also different in the two parasites (Fig. 8).

While in *Gastrocotyle* the posterior hooks retain their oncomiracidial size and form without any alteration throughout development, in *Pseudaxine*, between the 5- and 12-clamp stages, the proximal regions of the posterior hooks disappear so that these hooks decrease in length from 19 μ to about 12 μ, and assume a different shape (Fig. 9*b*). The posterior hooks of *Pseudaxine* persist in this reduced form throughout adult life. While the reduction in size of the posterior hooks of *Pseudaxine* is taking place, i.e. between the 5- and 12-clamp stages, the postero-lateral hooks are lost completely.

Whereas the positions relative to each other of the oncomiracidial and post-oncomiracidial hooks remain constant throughout life in *Gastrocotyle*, in *Pseudaxine* the 'anchor-bearing lappet' elongates considerably so that in the adult the surviving oncomiracidial posterior hooks and the post-oncomiracidial hooks are relatively further apart (Fig. 7*b*).

Other features of the larval development of *Pseudaxine* are generally similar to those of *Gastrocotyle*.

DISCUSSION

Both larvae remain bilaterally symmetrical for a considerable period of post-oncomiracidial development, sufficient for the acquisition of buccal suckers and a new pair of large hooks. It is possible that this period could be used to investigate experimentally the factors, environmental (unilateral incidence of the gill-ventilating current, see Llewellyn, 1957*b*) or genetical, which determine the side of the subsequent asymmetrical development of the clamps.

In both species a pair of relatively large hooks develops in the first post-oncomiracidial stage. There is no trace of these in the newly hatched larvae. Bychowsky (1957) expressed the opinion that the corresponding hooks in the Microcotylidae and Mazocraeidae were not homologous with any of the six pairs of hooks present in the larvae of the majority of diclidophorideans, and, quite independently, I referred to such hooks in *Microcotyle labracis* not as larval hooks, but as 'primordia of adult hooks' (Llewellyn, 1957*a*). The present study supports the view that these 'large hooks' are post-oncomiracidial, but since they are relatively most prominent and have reached their definitive size during early larval development, namely, after the loss of the oncomiracidial lateral hooks, and before the development of the clamps of the adult, these hooks are probably best regarded as essentially *larval* features. Their appearance during the embryonic development of *M. labracis* is probably no more than an example of the kind of precocious development met elsewhere in polyopisthocotylineans, e.g. the appearance in the *embryo* of *Diplozoon paradoxum* of the first pair of clamps of the adult (Zeller, 1872).

The finding of larval and immature stages of *Gastrocotyle* and *Pseudaxine* in May, but of only adults in July and August, suggests that a seasonal rhythm is present in the reproductive cycle of these parasites.

The recovery from hosts of over two years of age of four immature specimens of *Pseudaxine* and 15 immature specimens of *Gastrocotyle*, one of which was a very early larva at the stage before any clamps had developed, indicates that it is not only 'young' (under two years old) *Trachurus* which are susceptible to infestation by these monogeneans, but older fishes also.

The main difference between the larval development of *Gastrocotyle* and *Pseudaxine* and those of *Microcotyle spinicirrus* as described by Remley (1942) and *Diclidophora denticulata* as described by Frankland (1955) is the survival of the 'hook-bearing lappet' with at least some of its hooks in the first two species, and its complete disappearance in the other two species.

I am happy to acknowledge the help I have received from the Director and Staff of the Plymouth Laboratory, and especially that given by Mr J. E. Green and Mr A. D. Mattacola.

SUMMARY

Larval forms of *Gastrocotyle trachuri* and *Pseudaxine trachuri* are common on *Trachurus trachurus*, especially on young fishes, at Plymouth in May, but only adult parasites are found in July and August.

The larval development of these monogeneans includes a bilaterally symmetrical post-oncomiracidial stage in which the most prominent adhesive organs are a pair of relatively large post-oncomiracidial hooks. Asymmetrical development begins with the formation of the principal adult adhesive organs, the clamps.

REFERENCES

BYCHOWSKY, B. E., 1957. *Monogenetic trematodes, their classification and phylogeny.* 509 pp. Academy of Sciences, U.S.S.R. Moscow: Leningrad.

FRANKLAND, H. M. T., 1955. The life history and bionomics of *Diclidophora denticulata* (Trematoda: Monogenea). *Parasitology*, Vol. 45, pp. 313–51.

LLEWELLYN, J., 1954. Observations on the food and gut pigment of the Polyopisthocotylea (Trematoda: Monogenea). *Parasitology*, Vol. 44, pp. 428–37.

—— 1956. The host specificity, micro-ecology, adhesive attitudes, and comparative morphology of some trematode gill parasites. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 113–27.

—— 1957*a*. The larvae of some monogenetic trematode parasites of Plymouth fishes. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 243–59.

—— 1957*b*. Host specificity in monogenetic trematodes. In: *First Symposium on Host Specificity among parasites of Vertebrates*, pp. 191–212. Neuchâtel.

REMLEY, L. W., 1942. Morphology and life history studies of *Microcotyle spinicirrus* MacCallum, 1918, a monogenetic trematode parasitic on the gills of *Aplodinotus grunniens*. *Trans. Amer. micr. Soc.*, Vol. 61, pp. 141–55.

ZELLER, E., 1872. Untersuchungen über die Entwicklung des *Diplozoon paradoxum*. *Z. wiss. Zool.*, Bd. 22, pp. 168–80.

J. mar. biol. Ass. U.K. (1959) 38, 469–476
Printed in Great Britain

DIGESTION IN SEA ANEMONES

By J. A. C. Nicol
The Plymouth Laboratory

(Plate I and Text-fig. 1)

A sea anemone, normally a passive-looking animal, reacts to suitable foodstuffs by a series of fairly complicated activities. When its tentacles encounter solid food there is, first of all, a discharge of cnidae, which poison living prey and adhere to the food mass. These cnidae are independent effectors, responding directly to external excitation (Pantin, 1942). Next, the tentacles clasp the food and bend towards the mouth; they push the food into the mouth, and the pharynx draws it down into the coelenteric cavity where it is digested (Pantin & Pantin, 1943).

The food bolus lying in the coelenteric cavity gradually disintegrates, and the particulate and soluble products of digestion are absorbed. The indigestible residue is finally expelled from the mouth. Batham & Pantin (1950) have recorded the phasic activities of *Metridium* following feeding: these include elongation of the column, peristaltic movements and, finally, defaecation and shrivelling after about 48 h. The faecal pellets which are extruded are covered with thick mucus.

According to Yonge (1931, 1937, 1954), some preliminary digestion of protein takes place extracellularly in coelenterates, whereas the digestion of carbohydrates and fats, and the final degradation of proteins are all carried out intracellularly. Previous workers have had difficulty in demonstrating an extracellular protease in actinians, since fluid drawn from the coelenteron usually possesses little or no proteolytic activity (Mesnil, 1901; Bodansky, 1924). Many lines of evidence show, however, that extracellular digestion does take place (Boschma, 1925; Yonge & Nicholls, 1930; Krijgsman & Talbot, 1953). There is, seemingly, a paradox between the low proteolytic activity of coelenteric fluid, and the demonstrated efficacy of coelenteric digestion, and some experiments were made to investigate certain aspects of digestive secretion in sea anemones.

METHODS AND EXPERIMENTS

Most of the observations and experiments were made on the anemone *Calliactis parasitica* (Couch). For *in vitro* studies mesenteric filaments, together with neighbouring regions of the mesenteries, were removed from anaesthetized sea anemones. Roughly weighed samples of these tissues were used for investigations of protease activity.

For estimating protease activity, the photoelectric method of Riggs & Stadie (1943) was used. In this method, changes in the turbidity of a suspension of homogenized boiled egg white during the course of digestion are measured in an absorptiometer. From suitable dilutions of the substrate, a curve is obtained relating concentration to density. The initial concentration is taken as 100%. Density readings made of the digest at suitable times are converted to concentrations by means of this curve. Concomitantly with the digests, blanks are run that contain fluid to be tested and preservative, but no substrate. Values for the blanks are subtracted from the digests. Digests were carried out at pH 7·4, using boric acid–borate buffer. This hydrogen-ion concentration was chosen somewhat arbitrarily after reviewing the literature. Previous workers have shown that the optimal pH for intracellular proteases and the hydrogen-ion concentrations of coelenteric fluids of diverse Anthozoa range from pH 6·7 to 8·75 (Yonge & Nicholls, 1930; Krijgsman & Talbot, 1953).

Digestive activity of extracts

Tests were made of the proteolytic activity of crude extracts of mesenteric filaments of *Calliactis*. Filaments from an animal were ground with sand, 10 ml. of water were added, and the suspension was centrifuged and filtered. Results are given in Table 1. The rate of digestion, computed as a monomolecular reaction, is shown in Text-fig. 1. The velocity constant, *k*, falls off with time. The extract contains a strong protease acting at pH 7·4.

Proteolytic activity of coelenteric fluid

Samples were drawn of coelenteric fluid of some anemones to test for proteolytic activity. At most a few ml. were obtained from each animal. Out of 14 specimens (2 *Tealia felina* and 12 *Calliactis parasitica*), only 1 (*Tealia*) showed slight digestive activity (substrate concentration reduced to 73% in 18 hours).

Rate of digestion of a meal in a normal animal

The rate at which *Calliactis* can digest a meal was investigated as follows. Animals were fed weighed pellets of denatured gelatine. At selected intervals animals were sacrificed, the residual pellets (if any) recovered and weighed. Kjeldahl analyses were made of the latter.

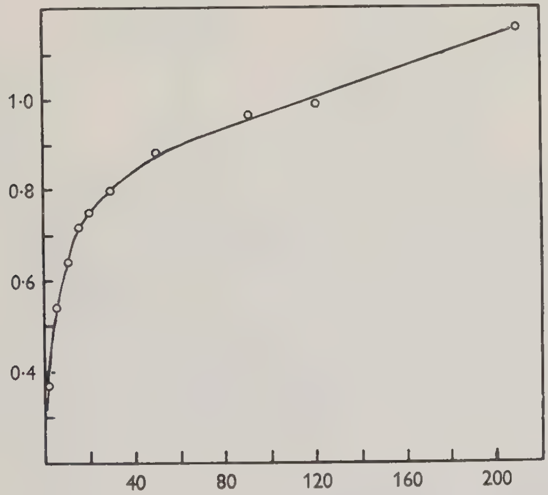
The pellets were prepared from a 10% solution of gelatine, denatured with formalin, and washed in sea water.

Fed sea anemones were sacrificed at 2, 4, 8, 16 and 24 hr. At least 3 animals were tested for each of these periods. Sea-water temperatures were about

TABLE 1. PROTEOLYTIC ACTIVITY OF EXTRACT OF MESENTERIC FILAMENTS OF *CALLIACTIS*

Period (time after mixing) (min)	Concentration of egg white (%)	k*
1	70·2	0·364
6	57·8	0·091
11	52·3	0·059
16	48·3	0·045
21	46·9	0·036
31	44·8	0·026
51	41·4	0·017
91	38·0	0·011
121	37·2	0·008
211	31·6	0·005

* $k = 2{\cdot}3/t \log_{10} C_0/C_t$ (proportion of protein hydrolysed per min.)



Text-fig. 1. Rate of digestion of egg white substrate by an extract of the mesenteric filaments of *Calliactis*. Abscissae, $2{\cdot}3 \times \log_{10} C_0/C_t$; ordinates, time in min after mixing extract and substrate.

TABLE 2. DIGESTION OF DENATURED GELATINE PELLETS BY *CALLIACTIS*

Digestion time (h)	No. of trials	Original wet weight (g)	Percentage of pellet digested	
			Basis wet weight	Basis N
2	5	0·766–1·035	44·5	45·4
4	5	0·592–0·847	65·9	79·1
8	8	0·906–1·022	53·9	48·9
16	5	0·663–1·019	76·5	80·7
24	3	0·687–1·013	98·7	98·6

12°–13° C. The results are summarized in Table 2. The gelatine blocks were about half digested in the first 8 h and completely digested in 24 h.

Progress of secretion with time

The temporal course of secretion *in vitro* was determined by the following procedure. In control experiments, 5 g of *Calliactis* tissue (isolated mesenteries and filaments) were placed in a vessel and covered with 25 c.c. of sea water. Samples of 1 c.c. were drawn from the supernatant fluid at the start and at 1 h intervals and were tested for protease activity. Room temperature was 18° C. The results appear in Table 3.

TABLE 3. PROTEASE ACTIVITY BY *CALLIACTIS* FILAMENTS *IN VITRO*

Time of drawing sample	Digest time	Final substrate concentration	k
0	23 h	100	—
1 h	22 h 10 min	96	$0{\cdot}030 \times 10^{-3}$
2 h	21 h 10 min	96	$0{\cdot}032 \times 10^{-3}$
3 h	20 h 5 min	87	$0{\cdot}117 \times 10^{-3}$
4 h	19 h 5 min	92	$0{\cdot}072 \times 10^{-3}$

Similar experiments were carried out with the addition of casein (B.D.H. soluble) to the supernatant fluid, to make 0·04%. Results are shown in Tables 4 and 5. Table 4 refers to an experiment in which 5 g of *Calliactis* tissue in 25 c.c. of fluid were used; Table 5, to an experiment with 10 g of tissue in 50 c.c. of fluid.

In the controls, protease levels showed a slight increase after 3 h. In the experimentals, exposed to an excitant (casein), proteolytic activity became marked after 4 h and remained at maximal plateau level for 10 h (duration of the experiment).

In vitro *studies with various excitants*

The results obtained in the experiments described in the previous section suggested the possibility of testing the relative efficacy of various excitants. Accordingly, a long series of experiments was carried out, using 1 g samples of mesenteric filaments in 5 c.c. of sea water. Excitatory substances tried were proteins, proteose, peptones, glutathione and many amino acids (0·02%). Test samples were drawn after 4 h (temp. 17°–19° C). Digest time was 19 h. Since the results were very variable, it is not proposed to describe them in detail. Controls (mean of 12 tests) showed a final substrate concentration of 86%; proteins (casein and egg albumen, 12 tests), 66–73%; peptones (bacteriological, 14 tests), 55–83%; proteose, glutathione, and amino acids (52 tests), 83–100%. Proteins and peptones were secretagogues, producing stronger secretory activity than that occurring in the controls.

TABLE 4*

Time of drawing sample	Digest time	Final substrate concentration	k
0 h	23 h 30 min	100	—
1 h	22 h 35 min	100	—
2 h	21 h 40 min	100	—
3 h	21 h	83	$0{\cdot}170 \times 10^{-3}$
4 h 20 min	19 h 40 min	66·5	$0{\cdot}343 \times 10^{-3}$

TABLE 5*

Time of drawing sample	Digest time	Final substrate concentration	k
0 h	23 h	95	—
2 h	23 h	66	$0{\cdot}298 \times 10^{-3}$
4 h	23 h	61	$0{\cdot}358 \times 10^{-3}$
6 h	23 h	59	$0{\cdot}383 \times 10^{-3}$
8 h	23 h	63	$0{\cdot}335 \times 10^{-3}$
10 h	21 h 5 min	62	$0{\cdot}370 \times 10^{-3}$

* Details in text.

DISCUSSION AND CONCLUSIONS

There is now clear evidence, both from previous observations and the present work, that a protease is secreted into the coelenteron of sea anemones. Earlier studies are reviewed by Boschma (1925) and Yonge (1931). Especially pertinent are the experiments of Jordan (1913), who enclosed fibrin in sacs of filter-paper and fed them to anemones (*Anemonia sulcata*). Digestion of the fibrin occurred and, since the mesenteric filaments could not come into contact with the food, he concluded that extracellular protease diffused into the sacs and caused hydrolysis of the fibrin. Krijgsman & Talbot (1953) fed *Pseudactinia flagellifera* with pieces of sponge soaked in meat extract. The sponges were removed, the juice expressed and tested for proteolytic activity (casein precipitation method and alcohol titration). With casein substrate, there was pronounced digestion after 3 h. With gelatine substrate, marked digestive activity was found after 3 h (increase of acidity measured by alcohol titration method of Waldschmidt–Leitz).

Studies dealing with excitation of digestive secretion in lower animals are rather infrequent. In the experiments of Krijgsman & Talbot (1953), cited above, the coelenteric fluid collected from anemones which were fed pieces of sponge containing meat extract showed pronounced proteolytic activity, but, as no similar experiments were recorded of feeding sponge alone, it is difficult to draw any conclusions concerning the stimulatory effect of meat extract on the secretory mechanism. Ishida (1936) collected coelenteric fluid from *Actinia mesembryanthemum* by cutting off the pedal disc; starved and fed animals were compared for proteinase activity. Three hours after feeding cooked egg white there was a marked increase in the proteinase ('tryptase') activity of the collected fluid.

Only a small amount of coelenteric fluid can be collected from sea anemones by suction or by cutting open the animals, and this fluid shows little proteolytic activity, even in fed animals (Bodansky, 1924). Yet there is secretion of protease, extracellular digestion proceeds rapidly and large food masses are quickly cleared. How are these apparent contradictions to be explained? Extracellular protease has been detected in fluids collected from the intact animal (*Hydra*, anemones), and secretion of protease has been demonstrated by using *in vitro* preparations (anemones). Krijgsman & Talbot (1953) believed that a food mass becomes coated with stiff mucus in the coelenteron of an anemone. The mucus coating is impregnated with proteinase, and forms a protective barrier against dilution by sea water; digestion takes place within the mucus coating. The particulate products of extracellular digestion are then phagocytized and digestion is completed intracellularly.

We have found no stiff masses of mucus about partially digested food boluses in *Calliactis*. In an expanded fed animal very little fluid can be collected from the base of the coelenteron. When fed sea anemones are quickly frozen (solid carbon dioxide in acetone) and cut open in the frozen state, the food bolus is found completely invested by mesenteric filaments at the base of the coelenteron, below the stomodaeum, and there is no fluid filled cavity in this region (Pl. I). This would explain why it is difficult to draw a sample of uncontaminated coelenteric fluid through the stomodaeum. Fluid which is obtained comes mostly from the upper regions of the coelenteron, or contains sea water drawn down the stomodaeum.

Mesenteric filaments of anemones are very active structures which move over, and closely adhere to the food bolus. Boschma (1925) and Yonge (1930) have made similar observations on corals (*Astrangia* and *Euphyllia*): they found that mesenterial filaments close quickly over plankton organisms, completely obscuring them. These filaments are in continual motion, one replacing another as soon as the first becomes gorged with food. The gastrozooids of *Physalia* behave in an analogous manner, closely enveloping the prey. Digestive enzymes are secreted, and partially digested food material passes up the lumina of the gastrozooids (Wilson, 1947).

The structure of the coelenteron favours enzymic activity. The food bolus is enveloped in a sac-like mass of mesenteric filaments which adhere closely to the surface of the food (Pl. I). Proteolytic secretion is stimulated by protein components in the food mass. The enzymes act on the food practically at the surface of the filaments, and suffer little dilution; as the food is dissolved and absorbed the filaments continue to press against the shrinking mass. This situation is in contrast to that found in many higher Metazoa, notably vertebrates, which discharge extracellular enzymes into large sacs or tubes, in the open lumina of which digestion takes place. The apparently feeble proteolytic activity of gastric fluids or *in vitro* samples, therefore, should be compared with the *in vivo* condition, in which the enzyme is concentrated in small volume, immediately over the food mass.

Corals and anemones digest food masses quickly. In the present experiments it was found that *Calliactis* would dissolve sticks of insoluble gelatine, weighing 0·6–1 g and containing 5–9 mg N, within 24 h (12° C). Yonge & Nicholls (1930) found that the corals *Fungia*, *Symphyllia* and *Favia* digested large plankton organisms (copepods, mysids, etc.) in 4–13 h (around 25° C). Masses of coagulated blood were digested by *Fungia* in 4 h.

It is generally believed that the extracellular protease of coelenterates hydrolyses some part of the protein fraction of the meal to polypeptides; the disintegrated food bolus is then phagocytized, and digestion is continued intracellularly (Yonge, 1937).

The mesenteric filaments of corals and anemones contain a powerful proteinase. This is regarded as being of the trypsin type (i.e. an endopeptidase acting in an alkaline medium on peptide linkages adjacent to arginine or lysine); it has the same pH optimum as extracellular protease, and may be identical with the latter (Krijgsman & Talbot, 1953). It would be reasonable to find the precursor of the extracellular protease in the secretory tissue. If digestion of proteins proceeds beyond the polypeptide stage, then other intracellular proteases must be involved.

SUMMARY

Proteolytic activity of *Calliactis parasitica* was investigated by a photoelectric method.

The filaments contain a strong protease; the coelenteric fluid shows little or no proteolytic action.

Gelatine pellets (up to 1 g wet weight and 9 mg N) were digested within 24 h (12° C).

Secretion by mesenteric filaments *in vitro* was followed. Proteins and peptones acted as secretagogues. Secretion reached a maximum in 4 h.

The mesenteric filaments closely invest the food bolus. Digestion, initiated by extracellular protease, takes place at the surface of the filaments, and partially decomposed food material is absorbed.

REFERENCES

Batham, E. J. & Pantin, C. F. A., 1950. Phases of activity in the sea-anemone, *Metridium senile* (L.), and their relation to external stimuli. *J. exp. Biol.*, Vol. 27, pp. 377–99.

Bodansky, M., 1924. Comparative studies of digestion. 3. Further observations on digestion in coelenterates. *Amer. J. Physiol.*, Vol. 67, pp. 547–50.

Boschma, H., 1925. On the feeding reactions and digestion in the coral polyp *Astrangia danae*, with notes on its symbiosis with zoöxanthellae. *Biol. Bull., Woods Hole*, Vol. 49, pp. 407–39.

ISHIDA, J., 1936. Digestive enzymes of *Actinia mesembryanthemum*. *Annot. zool. jap.*, Vol. 15, pp. 285–305.

JORDAN, H., 1913. *Vergleichende Physiologie wirbelloser Tiere*. 1. *Die Ernährung*. Jena: Fischer.

KRIJGSMAN, B. J. & TALBOT, F. H., 1953. Experiments on digestion in sea-anemones. *Arch. int. Physiol.*, Vol. 61, pp. 277–91.

MESNIL, F., 1901. Recherches sur la digestion intracellulaire et les diastases des Actinies. *Ann. Inst. Pasteur*, Vol. 15, pp. 352–97.

PANTIN, C. F. A., 1942. The excitation of nematocysts. *J. exp. Biol.*, Vol. 19, pp. 294–310.

PANTIN, C. F. A. & PANTIN, A. M. P., 1943. The stimulus to feeding in *Anemonia sulcata*. *J. exp. Biol.*, Vol. 20, pp. 6–13.

RIGGS, B. C. & STADIE, W. C., 1943. A photoelectric method for the determination of peptic activity in gastric juice. *J. biol. Chem.*, Vol. 150, pp. 463–70.

WILSON, D. P., 1947. The Portuguese man-of-war, *Physalia physalia* L., in British and adjacent seas. *J. mar. biol. Ass. U.K.*, Vol. 27, pp. 139–72.

YONGE, C. M., 1931. Digestive processes in marine invertebrates and fishes. *J. Cons. int. Explor. Mer*, Vol. 6, pp. 175–212.

—— 1937. Evolution and adaptation in the digestive system of the Metazoa. *Biol. Rev.*, Vol. 12, pp. 87–115.

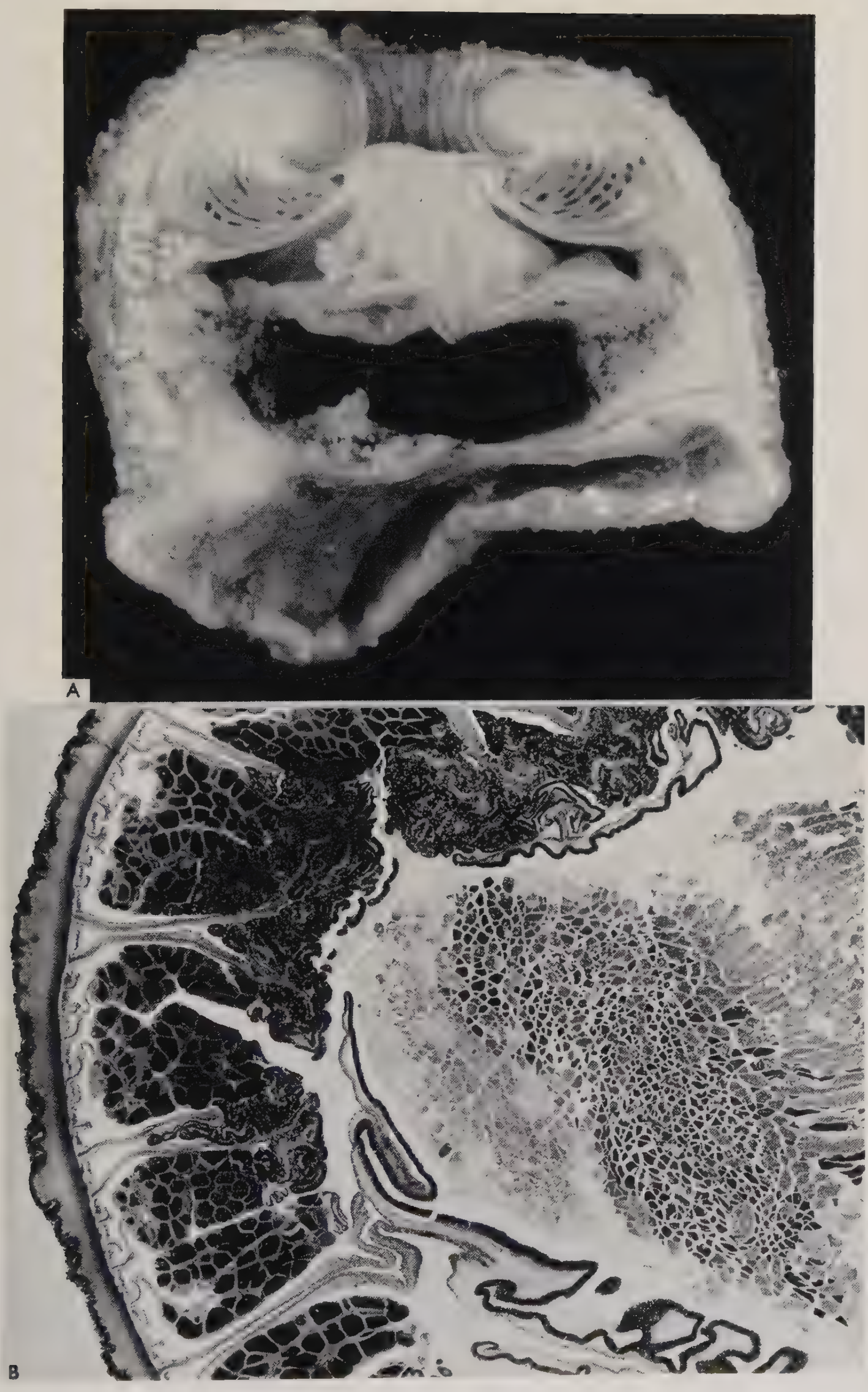
—— 1954. Physiological anatomy of the alimentary canal in invertebrates. *Tabul. Biol., Berl.*, Vol. 21, Pt. 3, pp. 1–24.

YONGE, C. M. & NICHOLLS, A. G. 1930. Studies on the physiology of corals. 2. Digestive enzymes. With notes on the speed of digestion. *Sci. Rep. Gr. Barrier Reef Exped.*, Vol. 1, pp. 59–81.

EXPLANATION OF PLATE I

A. A frozen *Calliactis*, cut vertically through the centre. The black rectangle, below the stomodaeum, is a gelatine pellet coloured red with carmine. It is closely invested by mesenteric filaments (× 5).

B. Horizontal celloidin section across the body of a fixed *Calliactis* that had been digesting a meal of plaice muscle. A mass of muscle lies in the coelenteron; note the change in density at the margin, due to partial digestion. Mesenteric filaments press closely against the muscle mass (a little separation has occurred during fixation) (× 12·5).



(*Facing p.* 476)

J. mar. biol. Ass. U.K. (1959) 38, 477–479
Printed in Great Britain

STUDIES ON LUMINESCENCE. ATTRACTION OF ANIMALS TO A WEAK LIGHT

By J. A. C. Nicol

The Plymouth Laboratory

(Text-fig. 1)

In order to evaluate the biological significance of luminescence, it is desirable to know how weak light affects the behaviour of marine animals, and what intensities they can see. Much information is available concerning the attractive power of bright lights, and light directed movements (see Verheijen, 1958, for example), but little for weak point sources. Weak coloured lights have been used by Baylor & Smith (1953) to trap freshwater arthropods.

In the present study, the reaction was tested of animals to a small light-lure, having the colour and intensity of animal luminescence. The light-lure was a conical light-guide, consisting of frosted glass 20 mm long, 4·4 mm in diameter at the base, and tapering to a point (Fig. 1). The light-lure was mounted in a light-proof case, containing a small light-bulb. A blue filter was placed between the bulb and the base of the light guide. The light which was emitted by the light-lure had a spectral range of 420–540 $m\mu$, and maximal emission at 475 $m\mu$. Intensities used were judged by eye to be equivalent to those observed in various marine animals, such as deep-sea fish with luminous barbels and deep-sea shrimp with photophores.

Animals were tested in a black tray, 51 × 41 × 10 cm (Fig. 1). This was divided into three compartments, left (*L*), centre (*C*) and right (*R*), separated from each other by clear plastic ('Perspex') partitions. The latter were > shaped, the apex pointing away from *C* to *L* or *R*. At the apex was a vertical slit, variable in width (1–3 cm), through which the animals could pass at will from the centre to the lateral compartments. The light-lure was hung vertically in one of the lateral compartments, 10 cm from a vertical slit. The tray was filled with sea water.

An experiment or test was carried out as follows. A batch of animals was dark-adapted for 1 h, following which they were placed in *C* and left in the dark for 1–5 h. Then the numbers of animals in the three compartments were counted. If the animals remained in *C* or distributed themselves equally among the *L*, *C* and *R*, it was concluded that they were not attracted by the light. If they tended to congregate in the lateral compartment containing the light, they were considered to be attracted by the latter.

Animals tried were small free-swimming crustacea and fish, easily obtained in inshore waters. The light intensity was varied with a rheostat.

Animals attracted by the light (protocols in Appendix 1) were: a decapod crustacean *Palaemonetes varians*, a mysid *Praunus neglectus*, and a copepod *Tigriopus fulvus*. Two other decapods *Crangon vulgaris* and *Pandalina brevirostris* were probably also attracted. Under the conditions of the experiment a decapod *Palaemon serratus* and a fish *Gobius flavescens* were not.

Praunus neglectus gave a clear-cut response, and was employed to determine the minimal intensity of the source to which the animals would respond (see Appendix 2 for protocols). This was $28 \cdot 7 \times 10^{-6}\,\mu W/cm^2$ receptor surface at 10 cm distance. Therefore, the animal responds to a flux of $29 \times 10^{-6}\,\mu W$ falling upon a flat surface of 1 cm square in the plane of its eye and at right angles to a line extending from the light-lure to the eye. In the dark-adapted state the eyes lighten, owing to withdrawal of iris-pigments ('superposition' condition). Let us assume that the eye of *P. neglectus*, diameter 0·68 mm, has an effective area of 0·36 mm² for incident light from a point-source. This is the area of a flat plane in the maximal diameter of the eye at right angles to the axis of the light-beam. Then, *P. neglectus* responds to $10 \cdot 5 \times 10^{-8}\,\mu W$ falling into each eye.

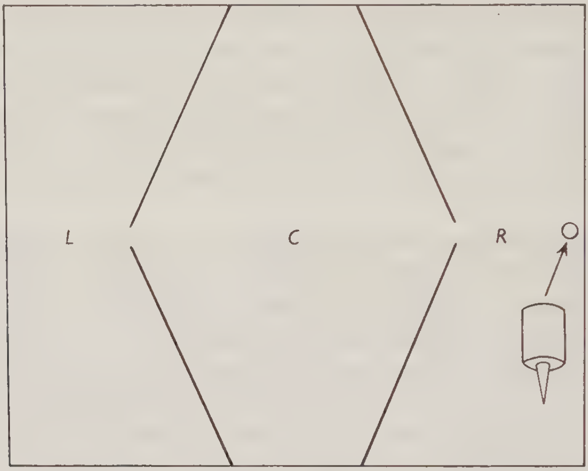


Fig. 1. Test-tray and light-guide in its holder (details in text).

It is not clear what should be regarded as the effective area of the dark-adapted ('superposition') compound eye of a malacostracan. The visual area involved is certainly less than the value just presented, since only a fraction of the ommatidia are affected by a directional light. In the superposition eye of *Lampyris*, some thirty neighbouring ommatidia may concentrate light from a point source upon a single rhabdome (Exner, in Wigglesworth, 1939).

Pirenne & Marriott (1955) have determined that a freshwater planarian, *Dendrocoelum lacteum*, responds to a radiant flux of 15×10^{-9} erg/sec falling into one eye of diameter 0·08 mm (i.e. $1{\cdot}5 \times 10^{-9}$ μW/eye). Values for radiant fluxes of animal-luminescences range from about 1×10^{-5} μW/cm² at 1 cm for a single radiolarian cell to 4×10^{-1} μW/cm² at 1 cm for *Pyrosoma* (Nicol, 1958).

SUMMARY

The attraction for animals of a small light-lure, emitting a feeble blue light, was tested in a multiple choice apparatus. *Palaemonetes varians*, *Praunus neglectus* and *Tigriopus fulvus* were attracted. The minimal intensity to which *P. neglectus* responded was 29×10^{-6} μW/cm² receptor surface.

REFERENCES

BAYLOR, E. R. & SMITH, F. E., 1953. A physiological light trap. *Ecology*, Vol. 34, pp. 223–5.

NICOL, J. A. C., 1958. Observations on luminescence in pelagic animals. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 705–52.

PIRENNE, M. H. & MARRIOTT, F. H. C., 1955. Light sensitivity of the aquatic flatworm *Dendrocoelum lacteum*. *Nature, Lond.*, Vol. 175, p. 642.

VERHEIJEN, F. J., 1958. The mechanisms of the trapping effect of artificial light sources upon animals. *Arch. néerl. Zool.*, Vol. 13, pp. 1–107.

WIGGLESWORTH, V. B., 1939. *The Principles of Insect Physiology*. London: Methuen.

APPENDIX

(1) *Test of animals with the light-lure*

Animal	Duration of experiment (h)	Numbers in compartments		
		L	*C*	*R*
Palaemonetes varians	1	1	3	11*
P. varians	1	2	0	13*
Palaemon serratus	1	5	3	7*
Pandalina brevirostris	5	6*	9	1
Praunus neglectus	1	1	7	7*
P. neglectus	2½	3	2	11*
Tigriopus fulvus	1	3	16	30*
Gobius ruthensparri	1	1	13	0*

(2) *Response of* Praunus neglectus *to various light intensities (test lasted 5 h)*

Intensity of source μW/cm² at 10 cm	Numbers in compartments		
	L	*C*	*R*
23×10^{-6}	7	2	7*
$77{\cdot}5 \times 10^{-6}$	1	3	15*
$48{\cdot}8 \times 10^{-6}$	3	5	12*
$36{\cdot}2 \times 10^{-6}$	9	2	12*
$31{\cdot}6 \times 10^{-6}$	7	3	17*
$28{\cdot}7 \times 10^{-6}$	6	1	11*
23×10^{-6}	8	1	9*

* Light-lure in this compartment.

J. mar. biol. Ass. U.K. (1959) 38, 481–491
Printed in Great Britain

ON THE SEXUAL BIOLOGY OF *PANDALUS BOREALIS* (CRUSTACEA DECAPODA)

II. THE TERMINATION OF THE MALE PHASE

By D. B. Carlisle

The Plymouth Laboratory

Pandalus borealis Krøyer is a protandric hermaphrodite. In some populations, e.g. that found off the Northumberland coast, the hermaphroditism is partial (Allen, 1959). That is to say only certain individuals undergo sex reversal, while others are primary females, never going through a male phase; all the males undergo sex reversal. In the terminology proposed by Carlisle (1959*a*) such populations exhibit partial obligatory protandric hermaphroditism. By contrast, the population of the Gullmarfjord, Sweden, which I have been investigating, appears to exhibit full obligatory protandric hermaphroditism: the population contains no primary females; all the females have passed through a male phase. The data presented in this paper have no bearing, therefore, on the factors regulating the production of primary females, since I have never encountered one.

In most of the southern populations of *P. borealis* (a useful summary is provided by Rasmussen, 1953), the eggs are carried for about 6 months. Since there is only one breeding season per year this means that copulation takes place between animals which are about 1½, 2½, or 3½ years old. The breeding season is somewhat prolonged, however, lasting up to 4 months. The evidence presented by Jägersten (1936), Rasmussen (1953) and Allen (1959) for different southern populations suggests that the male is not mature at 6 months old and cannot therefore breed until about 1½ years old when it is approximately 90 mm long. A few individuals may already by the end of this breeding season be functioning as females. By the next breeding season, when the animals are 2½ years old and measure about 110 mm, the great majority have reversed sex and copulate as females. A year later, at about 140 mm, they copulate again as females. Egg laying follows almost immediately upon copulation, in contrast to the condition found in Reptantia, where the interval may be as long as 6 months. According to Allen (1959) there are in the Northumberland population normally five moults between the fully functional male phase and the assumption of the breeding dress of the female; the last of the five moults is a moult of copulation. In the population of the Gullmarfjord the number of moults appears to be four, giving three intermoults between the male and the functional female states. In some individuals however, especially at certain seasons of the year, this may be cut to two intermoults or even to one; in experimental conditions a single individual has been observed to complete the change from male to female at one moult and breed as both male and female in one season. An individual taking only two moults (one intermoult) for the completion of sex reversal will also breed in both sexes in the one season under natural conditions; this is rare. The sex reversal takes place in the Gullmarfjord population at between 1½ and 2½ years old. All individuals seem to partake in the breeding season as males in their first season of maturity, all take part as females in their second season; some few may complete sex reversal in time to partake as females in the first season, after having earlier in the same season functioned as males.

In the first paper of this series (Carlisle, 1959*b*) I have described incretory organs which I have reason to believe may exert a controlling influence on the reversal of sex. In this paper I shall be concerned only with influences which bear upon the first part of the sex reversal—the loss of the male characters. For the purposes of these observations all individuals as far as possible were observed through one moult after experimental interference. Results are expressed in terms of loss or otherwise of a functional male condition after this one moult.

EXPERIMENTAL DATA

Vasectomy

Effective vasectomy may be achieved in a prawn by blocking the genital openings with small pellets of plasticine, soft wax, dental cement or cold-cure araldite. It is a commonplace that the operation performed on mammals (by ligating the vas deferens), besides leading to sterility, often leads also to impotence and to loss of libido (e.g. in the tom cat). In my experience the same is also true of prawns, including both *Pandalus borealis* and *Palaemon* (= *Leander*) *serratus*. This is most easily seen in the behaviour. As described by Höglund (1943), Burkenroad (1947) and Forster (1951), a ripe female which has just moulted, i.e. one which has just undergone the moult of copulation, proves attractive to males. Forster states of *P. serratus*: 'If an "attractive" female is placed in a tank with males nothing happens until the antenna of the male touches some part of the female. At once the male's behaviour alters, he swims very rapidly in no particular direction, often making small circles, until he again makes contact....' Essentially the same behaviour characterizes *Pandalus borealis*: the male, once he has touched an attractive female with his antenna, swims rapidly in circles, then with quick sharp motions, once he has found the female again, palpates her all over with his chelae. Copulation normally follows, but if several males are in a tank there may be some fighting.

When an attractive female is offered to 'vasectomized' males the response depends on the interval which has elapsed between the operation and the offer. No 'vasectomized' males were used until 48 h had elapsed, to allow time for postoperative recovery. Controls were animals in which the pellet was first inserted into each genital opening and then removed.

Up to 5 days after the operation the response of the 'vasectomized' males to the proffered females was normal, even including the fighting between rival suitors. Copulation was attempted but never completed. Six offerings were made to a tank containing nineteen males between 2 and 5 days after the operation. Each female was then removed after the failure at copulation and offered to a similar tank containing twenty controls. In each of these offerings copulation was completed satisfactorily.

Between 5 and 10 days post-operatively the interest exhibited by the operated males dropped progressively. In this interval I was able to offer them seven attractive females. The first of these on the sixth post-operative day elicited all the earlier part of the response without, however, any attempt at copulation. On the tenth day the offer of an attractive female elicited the swimming-in-circles response in about half the animals, but no further response. By the twelfth day an attractive female elicited no response. Every one of these females completed copulation successfully with one of the control animals. One operated animal moulted on the third day after the operation, shedding with the old shell the blocking pellets. The new shell was that of a fully functional male. Five days after the moult an attractive female was offered to him and they completed copulation satisfactorily. Histological examination after this event revealed a normal testis with spermatogenesis continuing, a normal vas deferens gland and a vas deferens still half full of sperm.

Other operated animals moulted from the seventh day onwards, and before my departure from the Kristineberg Laboratory nine had moulted (besides the one mentioned above), together with seven of the controls. All nine of the operated animals after moulting showed male appendages characteristic of the first non-sexual inter-moult, with reduction of the appendix masculina and loss of its setae. All of them at moulting shed the plug from the genital ducts. Histological examination revealed that spermatogenesis had ceased in these animals, the vas deferens was normal, but the vas deferens gland, though not characteristic of a normal first stage non-sexual animal, was greatly reduced and showed many pycnotic nuclei. Of the controls which moulted, only one passed into the non-sexual phase, and histologically it showed the conditions characteristic of this phase, with loss of the massive portion of the vas deferens gland, retaining only the strand. The other controls remained as functional males and retained the histological structures appropriate to this stage.

The conclusion is inevitable that occlusion of the spermduct in *P. borealis* causes sterility, loss of libido, and degeneration of the male genital system, and promotes the assumption of the non-sexual condition at the succeeding moult.

Eyestalk removal

This operation has been shown in other species of Natantia to have a profound effect on the development of the ovary and in particular on vitellogenesis (Panouse, 1943–6; Carlisle, 1953). The operation is followed by the initiation or acceleration of ovarian development and by vitellogenesis. The same is true of crabs (Brown & Jones, 1949), and in male crabs eyestalk removal is followed by a rapid increase in weight of the testes and vasa deferentia (Démeusy, 1953). It might be expected, therefore, that eyestalk removal would have some influence on the change from male to female in a protandric hermaphrodite. On the other hand, in the protandric hermaphrodite *Lysmata seticaudata* I have found neither eyestalk removal nor injection of eyestalk extracts to have any significant effect upon the weight of the testis or of the vas deferens (Carlisle, 1954). Tables 1 and 2 show that the same is true of *P. borealis*. The animals used in these experiments were all mature, sexually active males. Under the conditions of the experiments neither eyestalk removal nor injection of whole eyestalk extracts had any influence upon the weight of the testicular portion of the gonad, nor had starving, while the weight of the vas deferens was affected significantly only by starvation, not by either of the endocrine disturbances. It seems unlikely, therefore, that any tissue within the eyestalk has any effect upon testicular development.

The same result is apparent when the effect on the numbers of individuals losing the external male characters is considered. During April about half the large males which moult (in the samples I have examined) lose their secondary and accessory male characters (93 out of 197 moults observed) and change to the non-sexual phase. Interference with the eyestalks did not in any way alter this proportion. The results are presented in Table 3. Neither eyestalk removal nor injection of eyestalk extracts had any effect on the numbers moulting to the non-sexual state.

The vas deferens gland

The degeneration of this gland shortly before the termination of the male phase made it seem plausible that this event might be the immediate cause of the loss of the male character, since it is known that removal of the gland in *Orchestia* leads to loss of maleness (Charniaux-Cotton, 1954, 1955, 1956). Preliminary attempts at removing the gland by surgical methods in *Palaemon* proved abortive, because of total mortality of all operated animals within 24 h. The use of radio cautery, however, met with some success in *Palaemon* with less than 90% mortality from the operation. The results of these experiments will be reported elsewhere. When the method was applied to *Pandalus borealis*, however, I met with no success at first, most of the animals dying even before completion of the operation, and the remainder within a few hours. By modifying my techniques I eventually achieved success with no more than 75% mortality, better even than the earlier results with *Palaemon*. The technique of operation which I adopted may therefore perhaps be usefully described in some detail.

On the stage of the microscope was mounted a heavy, silver-plated brass plate with a central rectangular hole 20 × 7 mm. This was connected to the earth of the radio cautery outfit and served as the neutral electrode. On the brass plate were mounted two containers, whose bottoms were formed by the plate itself, in which could be placed crushed ice. These were mounted at the front and back of the stage to leave room for the hands at the sides. The source of illumination was filtered through copper sulphate solution to remove the infra-red rays. Before operation each animal was refrigerated at -1° C for 30 min. The fingers of my left hand were repeatedly cooled in ice water throughout the operation and were used only for holding and orientating the animal. By transmitted light it is just possible to distinguish the position of the vas deferens and of the gland in the intact animal. With the active electrode of the radio-cautery apparatus (a nickel-chrome (80/20) wire, $40\,\mu$ diameter, mounted in a glass holder) and with the apparatus turned to a rather high power I burned a hole near the base of the fifth walking leg immediately over the visible gland. The power was then reduced, the electrode inserted into the gland and the massive portion destroyed on one side. The animal was then immediately returned to the dish of ice-cold sea water and allowed to recover in the constant temperature room maintained at 4° C where the stock of animals was kept. Provided my left hand was kept cold and the operation was completed within about 2–3 min this stage resulted in negligible mortality. The other side of each animal was operated in like manner 24–48 h later. The longer period resulted in less deaths, which in my best series amounted to no more than 50%, while with a period between the two operations of only 24 h, even under the best conditions of cooling, deaths were usually more than 80%. Those animals which survived the first 48 h after the completion of the double operation showed a death rate not significantly different from unoperated controls.

It must be stated that in these experiments only the massive portion of the gland was destroyed, not the strand which runs the entire length of the vas deferens. Destruction has been checked histologically in all specimens. The control animals had the hole burned in the shell over the vas deferens gland, without the destruction of the gland itself; it was apparent that this part of the operation was the prime cause of the heavy mortality, since the deaths in this control group of animals almost equalled those in the operated group.

The operations to be reported here were all performed during the month of September, a few weeks before the start of the mating and egg-laying season or as it was just beginning. In over 300 moults observed amongst large mature males during this period not a single individual was observed to lose the male characters, while a sample of 2000 animals contained only two specimens in the inter-sexual condition. It is abundantly clear that sex reversal does not normally take place at this time of the year—all individuals seem to enter upon the mating season either as fully functional males or fully functional females. In the females vitellogenesis was well advanced and ovaries dark green. Any augmentation of the sex reversal is therefore most evident at this season.

The results of the operations in terms of loss of external male characters are presented in Table 4, and in terms of testis weight in Table 5. It is obvious that destruction of the massive portion of the vas deferens gland has led to degeneration of the testis and to loss of secondary and accessory male characters.

Examination of sections of the testes of these animals and of the sperm ducts provided additional confirmation of the degeneration of the former. The seminiferous tissue was shrunken and no sign of spermatogenesis could be seen. The sperm ducts by contrast were little altered and remained full of sperm.

A few days after the start of the above experiment a further series of eighty males was operated. Of these thirty-eight survived the operation and were used for injection experiments. Half received an intravenous injection of 0·15 ml. of distilled water 48 h after the completion of the operation and further similar injections 3 and 6 days later; these served as the controls. The other half had a similar series of injections of extract of vas deferens gland. This extract was prepared by homogenizing the fresh glands in distilled water in a Potter all-glass homogenizer. At each injection each animal received the extract of the glands from one individual. The animals were killed nine days after the first injection, 11 days after the end of the operation. The results are presented in Tables 6 and 7. The effects of the destruction of the vas deferens gland are partially but significantly countered by the injection of the extracts. Histological examination of the testes of these animals confirms that although they are somewhat degenerate with shrunken seminiferous tissue and absence of spermatogenesis, yet the shrinkage is not nearly so marked as in the uninjected controls.

DISCUSSION

The experiments reported in this paper make it plain that the hormones of the eyestalks have little or nothing to do with the loss of the male characters at the termination of the male phase in *Pandalus borealis*. On the other hand, it seems probable that this event is in large measure directly controlled by the secretions of the vas deferens gland. The gland degenerates in animals which are approaching the end of the male phase, its extirpation has resulted experimentally in the premature termination of the male phase at a time of year when this does not normally happen, and injection of extracts of the gland have partially prevented this result of operation. It seems probable also that the similar results which followed occlusion of the male aperture may in fact be ascribed to interference with this gland. Certainly it is most likely that some hormonal influence is involved since such occlusion results also in behavioural changes.

Such a conclusion about the importance of the role of the vas deferens gland does not of course preclude the possibility, or indeed the probability, that other endocrine organs are playing a part. Allen (1959) has implicated the follicle cells of the testis, for example, while it seems *a priori* probable that the endocrine complex of the protocerebrum might be exerting an over-riding endocrinokinetic control (Carlisle & Jenkin, 1959).

Despite such considerations, however, it seems to me that the immediate cause of the termination of the male phase is the degeneration of the vas deferens gland which was described in the first paper of this series (Carlisle, 1959). Other endocrine factors may exert a modifying influence or may operate through the vas deferens gland.

I should like to acknowledge the friendly assistance afforded me in this work by the former director, Dr G. Gustafson, and the staff of K. Svenska Vetenskapakadamien Kristinebergs Zoologiska Station.

SUMMARY

In the Gullmarfjord population of *Pandalus borealis* all individuals seem to be protandric hermaphrodites; no primary females have been seen by me in the years 1956, 1957 and 1958. Bilateral destruction of the vas deferens gland in mature males is followed by degeneration of the testes and by loss of the external male characters at the next moult, so that the animal moults into the non-sexual condition characteristic of the first stage of the normal sex reversal. These two effects of destruction of the gland have been partially prevented by injection of extracts from fresh glands. The same results follow occlusion of the male openings by means of pellets of cement. It is surmised that this is a result of damage to the vas deferens gland. Such occlusion of the vasa deferentia is followed also by loss of libido. Eyestalk removal and injection of eyestalk extracts have no significant effect on the testis, vasa deferentia, or rate of loss of male characters. Prolonged starvation results in loss of weight of the sperm-filled vasa deferentia, but has no noticeable effect on testis weight or histology. It is concluded that the immediate cause of the termination of the male phase in *P. borealis* is the degeneration (and consequent cessation of endocrine activity) of the vas deferens gland which always precedes this event.

REFERENCES

Allen, J. A., 1959. On the biology of *Pandalus borealis* Krøyer, with reference to a population off the Northumberland coast. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 189–220.

Brown, F. A. & Jones, G. M., 1949. Ovarian inhibition by a sinus-gland principle in the fiddler crab. *Biol. Bull., Woods Hole*, Vol. 79, pp. 228–32.

Burkenroad, M. D., 1947. Reproduction activities of decapod Crustacea. *Amer. Nat.*, Vol. 131, pp. 392–8.

Carlisle, D. B., 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). V. The ovarian inhibiting hormone and the hormonal inhibition of sex-reversal. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 435–47.

—— 1954. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). VIII. The lack of influence of eyestalk ablation and of injection of eyestalk extracts on testicular weight and degree of development of the male genital ducts. *Pubbl. Staz. zool. Napoli*, Vol. 25, pp. 241–5.

—— 1959*a*. Sexual differentiation in Crustacea Malacostraca. *Mem. Soc. Endocrinol.*, No. 7 (in the Press).

—— 1959*b*. On the sexual biology of *Pandalus borealis* (Crustacea Decapoda). I. Histology of incretory elements. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 381–94.

Carlisle, D. B. & Jenkin, P. M., 1959. Terminology of hormones. *Nature, Lond.*, Vol. 183, pp. 336–7.

Charniaux-Cotton, H., 1954. Découverte chez un Crustacé Amphipode (*Orchestia gammarella*) d'une glande endocrine résponsable de la différenciation de caractères sexuels primaires et sécondaires mâles. *C.R. Acad. Sci., Paris*, T. 239, pp. 780–2.

—— 1955. Le déterminisme hormonale des caractères sexuels d'*Orchestia gammarella* (Crustacé Amphipode). *C.R. Acad. Sci., Paris*, T. 240, pp. 1487–9.

—— 1956. Déterminisme hormonal de la différenciation sexuelle chez les Crustacés. *Ann. biol.*, T. 32, pp. 371–98.

Démeusy, N., 1953. Effets de l'ablation des pédoncules oculaires sur le développement de l'appareil génital mâle de *Carcinus maenas* Pennant. *C.R. Acad. Sci., Paris*, T. 236, pp. 974–5.

Forster, G. R., 1951. The biology of the common prawn, *Leander serratus* Pennant. *J. mar. biol. Ass. U.K.*, Vol. 30, pp. 333–60.

Höglund, H., 1943. On the biology and larval development of *Leander squilla* (L.) forma *typica* de Man. *Svenska Hydrograph. Biol. Komm. Skr.*, N.S.: Biol. Bd. 2, pp. 1–43.

Jägersten, G., 1936. Über die Geschlechtsverhältnisse und das Wachstum bei *Pandalus*. *Ark. Zool.*, Bd. 28, A, No. 20, 26 pp.

Panouse, J. B., 1943. Influence de l'ablation du pédoncule oculaire sur la croissance de l'ovaire chez la crevette *Leander serratus*. *C.R. Acad. Sci., Paris*, T. 217, pp. 553–5.

—— 1944. L'action de la glande du sinus sur l'ovaire chez la crevette *Leander*. *C.R. Acad. Sci., Paris*, T. 218, pp. 293–4.

—— 1946. Recherches sur les phénomènes humoraux chez les Crustacés. L'adaptation chromatique et la croissance ovarienne chez la crevette *Leander serratus*. *Ann. Inst. océanogr., Monaco*, T. 23, pp. 33–70.

Rasmussen, B., 1953. On the geographical variation in growth and sexual development of the deep sea prawn. *Rep. Norweg. Fish. Invest.*, Vol. 10, No. 3, 210 pp.

TABLE 1

Group	Treatment	No. in group	A	B
1	Intact. Fed *ad lib.*	27	3·157 ± 0·432	7·710 ± 0·554
2	Intact. Starved	26	3·263 ± 0·391	7·419 ± 0·516
3	Both eyestalks removed. Fed *ad lib.*	23	3·315 ± 0·302	7·895 ± 0·600
4	Both eyestalks removed. Starved	17	3·240 ± 0·611	7·314 ± 0·708

All animals were killed 15 days after the start of the experiment; animals dying before the close are not included. The animals which are recorded as 'starved' obtained a little food by scavenging in the tank.

A, mean weights and standard deviations (mg) of pairs of testes; B, mean weights and standard deviations (mg) of pairs of vasa deferentia.

ANALYSES OF VARIANCE

Nature of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Testis weights				
Operation	1	0·122	0·122	N.S.
Feeding	1	0·013	0·013	N.S.
Interaction	1	0·228	0·228	N.S.
Error	89	16·292	0·183	—
Total	92	16·654	—	—

$s = 0{\cdot}428$.

Nature of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Vas deferens weights				
Operation	1	0·144	0·144	N.S.
Feeding	1	4·064	4·064	< 0·001
Interaction	1	0·172	0·172	N.S.
Error	89	26·197	0·294	—
Total	92	30·576	—	—

$s = 0{\cdot}543$.

N.S. = not significant.

TABLE 2

Group	Treatment	No. in group	A	B
5	Intact. Injected distilled water. Fed *ad lib.*	23	3·258 ± 0·408	7·777 ± 0·532
6	Intact. Injected distilled water. Starved.	25	3·250 ± 0·439	7·431 ± 0·517
7	Intact. Injected eyestalk extract. Fed *ad lib.*	20	3·201 ± 0·397	7·938 ± 0·505
8	Intact. Injected eyestalk extract. Starved.	22	3·111 ± 0·515	7·505 ± 0·539
9	Both eyestalks removed. Injected distilled water. Fed *ad lib.*	18	3·330 ± 0·430	7·818 ± 0·590
10	Both eyestalks removed. Injected distilled water. Starved.	16	3·090 ± 0·319	7·475 ± 0·611
11	Both eyestalks removed. Injected eyestalk extract. Fed *ad lib.*	20	3·210 ± 0·592	7·793 ± 0·58
12	Both eyestalks removed. Injected eyestalk extract.	18	3·227 ± 0·577	7·390 ± 0·680
		162		

All animals were killed 13 days after the start of the experiment; animals dying before the close are not included. The animals which are recorded as starved obtained a little food by scavenging in the tank. All animals were injected at the start and again on the sixth day with 0·05 ml. either of distilled water or of a whole eyestalk extract made by grinding fresh male eyestalks with sand and distilled water and centrifuging; the extract was equivalent to 40 eyestalks/ml.

A, mean weights and standard deviations (mg) of pairs of testes; B, mean weights and standard deviations (mg) of pairs of vasa deferentia.

ANALYSES OF VARIANCE

Nature of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Testis weights				
Operation	1	0·001	0·001	N.S.
Feeding	1	0·269	0·269	N.S.
Injection	1	0·155	0·155	N.S.
Interaction-OF	1	0·007	0·007	N.S.
Interaction-FI	1	0·063	0·063	N.S.
Interaction-OI	1	0·170	0·170	N.S.
Triple interaction	1	0·255	0·255	N.S.
Error	154	32·920	0·214	—
Total	161	33·840	—	—

s = 0·462.

Nature of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Vas Deferens Weights				
Operation	1	0·022	0·022	N.S.
Feeding	1	5·856	5·856	< 0·001
Injection	1	0·059	0·059	N.S.
Interaction-OF	1	0·095	0·095	N.S.
Interaction-FI	1	0·036	0·036	N.S.
Interaction-OI	1	0·304	0·304	N.S.
Triple interaction	1	0·034	0·034	N.S.
Error	154	43·119	0·280	—
Total	161	49·491	—	—

s = 0·529.

N.S. = not significant.

TABLE 3. THE LOSS OF THE EXTERNAL MALE CHARACTERS AT THE MOULT FOLLOWING EYESTALK REMOVAL OR INJECTION OF EYESTALK EXTRACT

Group	Eyestalks removed	Injected	No. of moults	No. losing male characters	Proportion losing male characters
14	−	−	197	93	0·472
15	+	−	94	43	0·457
16	−	+	87	40	0·460
17	+	+	103	49	0·476
Total			481	225	0·468

$P \simeq 0{\cdot}7.$

TABLE 4. THE LOSS OF EXTERNAL MALE CHARACTERS AT THE MOULT FOLLOWING DESTRUCTION OF THE VAS DEFERENS GLAND

	Operated	Controls
Retaining male characters	0	9 (11)
Losing male characters	7 (11)	0

$P < 0{\cdot}001.$

Moults 4 or more days after the completion of the operation; two earlier moults in the control group are omitted. The figures in parentheses include animals which were sufficiently advanced in proecdysis at the end of the experiment for the new appendages to be dissected free of the old integument.

TABLE 5. TESTIS WEIGHT 12 DAYS AFTER VAS DEFERENS GLAND DESTRUCTION

	No. surviving	Testis weight (mg) and standard deviation
Gland destroyed	16	2·110 ± 0·225
Controls	20	3·725 ± 0·371

$P < 0{\cdot}001.$

TABLE 6. THE LOSS OF EXTERNAL MALE CHARACTERS AT THE MOULT FOLLOWING DESTRUCTION OF THE VAS DEFERENS AND INJECTIONS OF EXTRACTS OF THE GLAND

	Injected	Controls
Retaining male characters	0	4 (5)
Losing male characters	6 (7)	3

$P = 0{\cdot}049, \quad P' = 0{\cdot}022.$

Moults 4 or more days after the completion of the operation. The figures in parentheses include animals which were sufficiently advanced in proecdysis at the end of the experiment for the new appendages to be dissected free of the old integument. P' is the probability including these animals, P that excluding them, utilizing only the actual moult figures.

TABLE 7. TESTIS WEIGHT 11 DAYS AFTER VAS DEFERENS GLAND DESTRUCTION FOLLOWED BY INJECTION OF EXTRACTS OF THE GLAND

	No. surviving	Testis weight (mg) and standard deviation
Injected	14	2·795 ± 0·230
Controls	15	2·011 ± 0·222

$P < 0{\cdot}001.$

J. mar. biol. Ass. U.K. (1959) 38, 493–506
Printed in Great Britain

ON THE SEXUAL BIOLOGY OF *PANDALUS BOREALIS* (CRUSTACEA DECAPODA)

III. THE INITIATION OF THE FEMALE PHASE

By D. B. Carlisle
The Plymouth Laboratory

(Text-figs. 1–2)

In the second paper of this series (Carlisle, 1959*b*) I have described the results of various experimental manipulations upon the termination of the male phase in the protandric hermaphrodite *Pandalus borealis* Krøyer. In this paper I shall be concerned with the effects of such interference upon the initiation of the female phase of the life history.

In a number of papers Panouse (1943–8) provided evidence, based upon extirpation experiments, that the sinus gland of the eyestalks of the common European prawn *Palaemon* (= *Leander*) *serratus* secretes a hormone which inhibits the development of the ovary and in particular vitellogenesis. His results were confirmed by Takewaki & Yamamoto (1950*a*, *b*), who worked upon the Japanese prawn *Paratya.* Since the above work was completed much evidence has accumulated that the sinus gland is not in itself an endocrine organ, but a neurohaemal organ (Carlisle & Knowles, 1953); that is to say it is the terminal organ of a neurosecretory system, whose main function is to release into the blood hormones produced elsewhere, within neurosecretory cells. It is generally (but not universally) accepted that the hormones stored in the sinus gland, and released into the blood there, are produced mainly within the neurosecretory cells of the ganglionic X organs (see discussion in Carlisle & Knowles, 1959). In agreement with this view I have found that, both in *Palaemon* (Carlisle, unpublished) and in the Mediterranean prawn *Lysmata seticaudata* (Carlisle, 1953*a*, *b*), it is possible to extract an ovary-inhibiting substance from the sinus gland and from the ganglionic X organ which lies in the medulla terminalis of the brain (see Carlisle, 1953*c*). This substance, after intravenous injection, produces involution of the developing ovary in the intact animal, or effectively counteracts the result of eyestalk ablation, so far as ovarian growth is concerned. Extirpation of the sinus glands alone leads to an increase in ovarian weight significantly less than does total eyestalk ablation—as indeed Panouse also found. Ablation of the ganglionic X organ of the medulla terminalis leads to a delayed onset of increase in ovarian size, but once this increase has begun it proceeds as rapidly as after total eyestalk ablation (Carlisle, unpublished). We may suppose that the delay is a result of the hormone stored within the sinus gland: once this has been utilized, then ovarian increase can begin. The sinus gland seems nevertheless to be necessary for the efficient release of the hormone, for its removal does lead to some increase in ovarian size, until a new sinus gland is regenerated from the stump of the X organ-sinus gland neurosecretory tract.

The evidence seems satisfactory that ovarian growth in these two species of prawns is inhibited by a hormone secreted by the X organ-sinus gland complex. This complex in *Pandalus* has been described in the first part of this series (Carlisle, 1959*a*). Unpublished data suggest that in *Palaemon*, during the juvenile phase, the ovary is inhibited by the action of this hormone; before the onset of maturity the secretion of the hormone ceases and the ovary begins to develop; ovulation succeeds and once more the ovary is inhibited by the hormone until the breeding season of the following year. There is thus an annual cycle in the secretion of the hormone once the animal is mature. Arvy, Echalier & Gabe (1954) have provided evidence that the immature gonad may be influenced by a secretion of the Y organ in the crab *Carcinus*, but this has not yet been confirmed in *Palaemon*, nor indeed in any natantian, nor do these authors suggest that this organ has any influence on the gonad after sexual maturity.

Whereas most of the Decapoda are normal bisexual creatures, three isolated genera have been found to show a functional sex reversal, at least in some of their species. The blind burrowing decapod *Calocaris macandreae* is known to show this phenomenon (Runnström, 1925; Balss, 1930); *Lysmata seticaudata* and *L. nilita* apparently show full obligatory protandric hermaphroditism, so that every male which survives long enough reverses sex to become a female, while there are no primary females in the population (Spitschakoff, 1912; Caroli, 1917; Dohrn, 1950; Dohrn & Holthuis, 1950; Carlisle & Dohrn, 1952, 1953; the terminology is that proposed by Carlisle, 1959*c*); certain species of the genus *Pandalus*, notably the deep-sea arctic prawn *P. borealis*, the subject of the present communication, *P. montagui* and *P. kessleri* are also protandric hermaphrodites (Berkeley, 1930; Jägersten, 1936; Rasmussen, 1953; Aoto, 1952) though according to Pike (1952) *P. bonnieri* is dioecious. In *P. montagui* and in *P. borealis* most populations show a variable percentage of primary females so that the hermaphroditism is partial but obligatory (Mistakidis, 1957; Allen, 1959). In some populations, however, including that upon which I have worked in the Gullmarfjord, Sweden, the hermaphroditism is full and there are no primary females in the population (Carlisle, 1959*b*). The analysis of the sex reversal is of course easier in such a population.

I have shown (Carlisle, 1953*a*, 1954) that the assumption of functional female form in *Lysmata seticaudata* is inhibited by a hormone of the X organ-sinus gland complex. I then suggested that it seemed probable that the hormone responsible for this inhibition might well be the ovary-inhibiting hormone. The evidence that I am about to present suggests that in *Pandalus borealis* also, the ovary is inhibited by a hormone of the X organ-sinus gland complex, that the assumption of the female form is also inhibited by a hormone of this complex, and that there is a high degree of correlation between the two actions.

EXPERIMENTAL DATA

Change of the apparent sex of the external accessory and secondary sexual characters can only take place in a crustacean when the animal moults, shedding the old shell and growing a new one of different dimensions and with differing proportions. In a prawn possessing both functional ovarian and functional testicular tissue the functional sex is that corresponding to the external characters, for even if the testis is producing sperm, the prawn cannot mate as a male if it lacks the copulatory appendages and if the vasa deferentia terminate blindly with no opening to the outside; nor can it mate as a female, though possessing a functional ovary, if it lacks the female apertures and the ovigerous hairs. A prawn which has not moulted during the period of observation has had no chance to modify its sexual condition. In all experiments reported here, therefore, only individuals which had moulted during the experimental period are included in the data. All animals which did not moult before the end of the experiment were disregarded.

OVARIAN INHIBITION

Effects of the X organ-sinus gland complex

Eight groups of animals were selected, four of females and four of large males. Two groups of each sex were left intact and two groups had the eyestalks extirpated. One group from each division received a single injection of 4 sinus gland equivalents of an extract prepared from the sinus glands of females. Table 1 summarizes the treatments accorded to the different groups together with the mean ovarian weight in each group after a period of ten days had elapsed. Table 2 supplies a summary of the analysis of variance of these data. It will be seen that the operation of eyestalk removal is followed by a very significant increase in ovarian weight, while the injection of the extract is followed by the reverse. In the females, when both the operation and the injection are performed upon the same group of animals (group 4), it is the operation which has the greater effect—the injection is evidently inadequate to counteract the effect of the operation. In the smaller males, however, which received the same dose of the extract as the larger females and hence a larger dose per unit body weight, the effects of the injection and of the operation have effectively cancelled each other out, so that the mean ovarian weight in group 8 is the same as in group 5.

It is to be noted in the table of analysis of variance (Table 2) that there is a highly significant entry for 'interactions'. This is not unexpected for of course the males and females are in different physiological states, and it is not to be expected that either the injection or the operation will have exactly the same degree of action on the ovarian weight in the two sexes. Moreover, since all individuals, whatever their size, received the same dose of extract,

TABLE 1. OVARIAN INHIBITION

Group	No. in group	Sex	Operated	Injected	Mean ovarian weight (mg)	Standard deviation	Standard error of mean
1	15	♀	−	−	34·79	9·58	2·56
2	11	♀	−	+	23·63	8·22	2·60
3	14	♀	+	−	123·71	30·33	8·41
4	10	♀	+	+	42·51	4·41	1·47
5	24	♂	−	−	19·58	6·95	1·45
6	25	♂	−	+	16·45	7·94	1·62
7	19	♂	+	−	27·86	8·49	2·00
8	27	♂	+	+	19·44	6·99	1·37

TABLE 2. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 1 (OVARIAN INHIBITION)

Source of variation	Degrees of freedom	Sum of squares	Probability
Sex	1	48,313	< 0·001
Operation	1	20,346	< 0·001
Injection	1	19,136	< 0·001
Interactions	4	45,918	< 0·001
Error	137	20,626	—
Total	144	154,339	—

$s^2 = 150{\cdot}59$. $s = 12{\cdot}25$.

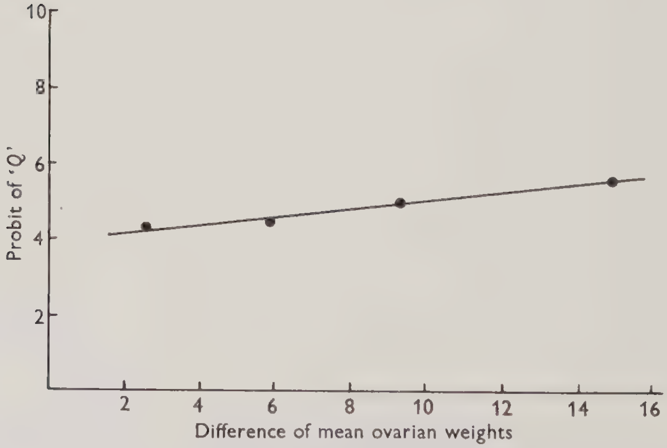


Fig. 1. The correlation in response to various treatments of the ovarian weight, with proportion becoming female.

whereas the operation would have, presumably, a uniform effect per individual, it might be expected that these two factors would interact. In fact, if the analysis of variance is carried further to separate the various possible interactions orthogonally, each of them is found to be significant. This, however, is irrelevant to the main argument. There is no doubt that eyestalk ablation leads to a rapid increase in ovarian weight and that injection of sinus gland extract prevents this wholly or in part, while in the intact animal such an injection is followed by involution of the ovaries. The case is clear for the hormonal inhibition of ovarian development by the X organ-sinus gland complex.

Effects of other organs and tissues

Complete selective extirpation of other tissues has not been attempted in this series of experiments. Removal of the eyestalk necessarily involves the ablation of other organs besides the sinus glands and the ganglionic X organs. Attempts at replacement therapy with tissues of the eyestalk other than these had no effect. In particular injections of extracts of the sensory pore X organ (*SPX*), of retroretinal tissue (*RT*) or of the medulla externa (*ME*) had no effect on ovarian weight in either males or females, intact or lacking eyestalks. Injections of extracts of the medulla terminalis ganglionic X organ (*MTGX*), however, acted like sinus gland extract injections. The data are summarized in Tables 3 and 4. The experiment was carried out in like manner with like dosages and times as in the first experiment reported above. In each group only the first twelve animals caught at the end of experiment were dissected and find inclusion in these tables. The remainder were discarded. It is abundantly clear that injection of extracts of the *SPX*, *ME* and *RT* are without any effect on ovarian weight in any of the conditions of the experiment.

The vas deferens gland

This gland, which has been shown to be necessary for maintaining the male phase (Carlisle, 1959*b*), is absent from the later non-sexual stages and from the female. Injection of extracts of the gland in dosages which had a profound effect upon the male (Carlisle, 1959*b*) had no effect upon oocytes that were undergoing vitellogenesis, or upon ovulation. Ovaries which were sectioned ten days after a single injection of vas deferens gland extract, however, showed the presence of cells which appeared to have the character of spermatogonia, while these were more clearly defined in animals which had had two such injections. Much more work is required upon this point, especially in view of the results of Charniaux-Cotton (1954) with *Orchestia*, and I will refrain from further comment until I have had the opportunity to experiment once again upon the vas deferens gland of *Pandalus*.

TABLE 3. OVARIAN INHIBITION

Group	No. in group	Sex	Operated	Injected *MTGX*	Injected *SPX*	Injected *ME*	Injected *RT*	Mean ovarian weight (mg)	Standard deviation
9	12	♀	–	–	–	–	–	35·01	8·4
10	12	♀	–	–	–	–	+	35·27	8·93
11	12	♀	–	–	–	+	–	34·20	9·01
12	12	♀	–	–	–	+	+	34·35	8·70
13	12	♀	–	–	+	–	–	36·07	8·25
14	12	♀	–	–	+	–	+	35·05	8·26
15	12	♀	–	–	+	+	–	34·76	8·78
16	12	♀	–	–	+	+	+	34·60	8·99
17	12	♀	–	+	–	–	–	23·14	8·21
18	12	♀	–	+	–	–	+	23·77	8·20
19	12	♀	–	+	–	+	–	23·09	7·95
20	12	♀	–	+	–	+	+	22·91	8·12
21	12	♀	–	+	+	–	–	25·05	8·88
22	12	♀	–	+	+	–	+	24·73	8·70
23	12	♀	–	+	+	+	–	23·68	8·76
24	12	♀	–	+	+	+	+	23·50	8·90
25	12	♀	+	–	–	–	–	120·01	30·24
26	12	♀	+	–	–	–	+	110·37	33·76
27	12	♀	+	–	–	+	–	130·25	31·09
28	12	♀	+	–	–	+	+	104·38	33·02
29	12	♀	+	–	+	–	–	114·46	27·89
30	12	♀	+	–	+	–	+	137·50	26·66
31	12	♀	+	–	+	+	–	90·92	27·84
32	12	♀	+	–	+	+	+	127·76	35·21
33	12	♀	+	+	–	–	–	44·26	7·22
34	12	♀	+	+	–	–	+	42·57	7·20
35	12	♀	+	+	–	+	–	39·20	7·91
36	12	♀	+	+	–	+	+	40·11	6·93
37	12	♀	+	+	+	–	–	39·34	7·29
38	12	♀	+	+	+	–	+	40·31	7·28
39	12	♀	+	+	+	+	–	42·41	6·91
40	12	♀	+	+	+	+	+	42·00	7·35
41	12	♂	–	–	–	–	–	18·75	7·04
42	12	♂	–	–	–	–	+	18·85	6·82
43	12	♂	–	–	–	+	–	19·34	6·64
44	12	♂	–	–	–	+	+	19·39	7·32
45	12	♂	–	–	+	–	–	19·42	7·49
46	12	♂	–	–	+	–	+	19·04	7·34
47	12	♂	–	–	+	+	–	19·58	7·14
48	12	♂	–	–	+	+	+	18·63	7·06
49	12	♂	–	+	–	–	–	15·82	7·03
50	12	♂	–	+	–	–	+	16·13	7·09
51	12	♂	–	+	–	+	–	16·60	6·88
52	12	♂	–	+	–	+	+	16·55	7·41
53	12	♂	–	+	+	–	–	15·90	6·97
54	12	♂	–	+	+	–	+	16·03	7·32
55	12	♂	–	+	+	+	–	16·07	7·58
56	12	♂	–	+	+	+	+	16·51	6·99
57	12	♂	+	–	–	–	–	27·83	7·95
58	12	♂	+	–	–	–	+	25·97	8·47
59	12	♂	+	–	–	+	–	25·96	8·99
60	12	♂	+	–	–	+	+	26·34	7·63
61	12	♂	+	–	+	–	–	26·51	8·60
62	12	♂	+	–	+	–	+	26·70	7·64
63	12	♂	+	–	+	+	–	26·03	7·70
64	12	♂	+	–	+	+	+	27·58	8·94
65	12	♂	+	+	–	–	–	19·91	7·00
66	12	♂	+	+	–	–	+	18·87	7·32
67	12	♂	+	+	–	+	–	18·92	7·56
68	12	♂	+	+	–	+	+	19·73	8·39
69	12	♂	+	+	+	–	–	19·21	7·34
70	12	♂	+	+	+	–	+	19·87	7·43
71	12	♂	+	+	+	+	–	20·03	8·09
72	12	♂	+	+	+	+	+	20·11	8·81

TABLE 4. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 3

Source of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Sex	1	219,882·8	219,883	≪ 0·001
Operation	1	146,440·1	146,440	≪ 0·001
MTGX	1	112,447·7	112,448	≪ 0·001
Interactions of Sex, op., *MTGX*	4	265,671·1	66,418	≪ 0·001
Other injections	3	372·8	124·3	N.S.
Other interactions	53	17,798·2	335·8	*c.* 0·01
Error	704	121,591·9	172·7	
Total	767	885,701·8		

s = 13·1. N.S., not significant.

ATTAINMENT OF THE FUNCTIONAL FEMALE STATE

Effects of the X organ-sinus gland complex

To determine the effects of the X organ-sinus gland complex upon the attainment of the functional female state animals in the intersexual condition were chosen. The individuals so chosen were allocated to groups at random. The animals were treated like those of groups 5–8 in the first experiment reported above, but three types of extract were used:

(1) Extract of the sinus gland of females, which at this season of the year (April) might be presumed to be moderately active in secreting ovary-inhibiting hormones;

(2) Extract of the sinus glands of young males, which might be presumed to be fully active in the secretion of the ovary-inhibiting hormone;

(3) Extract of the sinus gland of old males and non-sexual individuals, which might be presumed to be relatively inactive in the ovary-inhibiting hormone.

The animals in the first experiment received an injection of type 1 and the data from groups 5–8 of this experiment will be considered here also. The treatments accorded to the various groups are summarized in Table 5, together with the numbers which did and which did not attain a functional female condition at the succeeding moult. Table 6 summarizes the statistical analysis of these data. It will be seen that eyestalk removal has led to a very significantly higher proportion of attainment than in the unoperated animals, while injection of sinus gland extracts has had the opposite effect. The most effective type of extract was type 2 and the least effective type 3 which had an insignificant effect. Perusal of Table 5 will show that an injection of type 1 or type 2 effectively counteracted the effects of the operation, affording adequate replacement therapy in this respect; statistical analysis confirms this impression. It is evident that the attainment of a functional female state from the non-sexual condition is inhibited by some hormonal emanation from the sinus gland.

TABLE 5. ATTAINMENT OF FEMALE STATE: TREATMENTS AND RESULTS IN THE VARIOUS GROUPS

Group	No. in group	Operated	Injected (type 1)	Injected (type 2)	Injected (type 3)	Number becoming female	Number remaining non-sexual
73	24	−	−	−	−	12	12
74	25	−	+	−	−	9	16
75	19	+	−	−	−	16	3
76	27	+	+	−	−	14	13
77	28	−	−	−	−	15	13
78	26	−	+	−	−	9	17
79	23	+	−	−	−	18	5
80	28	+	+	−	−	13	15
81	30	−	−	−	−	14	16
82	23	−	−	+	−	5	18
83	22	+	−	−	−	17	5
84	31	+	−	+	−	12	19
85	29	−	−	−	−	15	14
86	31	−	−	−	+	15	16
87	31	+	−	−	−	24	7
88	30	+	−	−	+	23	7

Effects of other organs and tissues

Once more no attempt has been made at complete selective extirpation of other organs. An experiment similar to that summarized in Tables 3 and 4, but utilizing non-sexual animals, made it plain that injection of extracts of the *MTGX* acted in precisely the same way as extracts of sinus glands upon the attainment of the functional female condition, while injection of extracts of the *SPX*, *ME* and *RT* had no significant effect. The results of this experiment will not be reported in full.

I could find no evidence that injection of vas deferens gland extract affected the attainment of the functional female state, but this requires further investigation.

THE CORRELATION BETWEEN OVARIAN GROWTH AND ATTAINMENT OF THE FUNCTIONAL FEMALE STATE

As a measure of the effectiveness of the various treatments in interfering with the normal rate of attainment of the female state the coefficient of association (Q) was calculated (Yule, 1900; Yule & Kendall, 1945). The values of Q are listed in Table 6 and repeated in Table 7. The differences in mean ovarian weight brought about in the same individuals by the same treatments were calculated, as a measure of the effectiveness of these treatments in interfering with ovarian weight; these values are also listed in Table 7. If the phenomena of hormonal inhibition of attainment of the female status and of hormonal inhibition of ovarian growth are not interconnected these values should show no significant regression one upon another, whereas if they are connected there should be a significant regression.

Now Q is a coefficient which has the limits $+1$ and -1, and the graph of a function which is constrained to lie between limits is of necessity sigmoid, hence some linearizing transformation is needed if we are to calculate a linear regression of Q upon the difference in mean ovarian weights. According to Finney (1952) there is little to choose between the various linearizing transformations of sigmoid curves especially over the middle of the range, when we do not know on *a priori* grounds which sigmoid to expect. It is possible that the logit transformation might give a closer fit, but for ease of computation the probit transformation was chosen. The expected probits corresponding to each value of Q are listed in Table 7. A regression equation of these expected probits (y), suitably weighted, upon the difference in mean ovarian weights (x), was then computed by the usual probit method (Finney, 1952). After appropriate weighting the equation obtained was

TABLE 6. ATTAINMENT OF FEMALE STATE: χ^2 AND COEFFICIENTS OF ASSOCIATION (Q) BETWEEN ATTAINMENT AND DIFFERENT TREATMENTS

Treatment	$\chi^2_{[1]}$	Probability	'Q'	Standard error of 'Q'
Operation	19·112	< 0·001	+0·412	0·083
Injection (type 1)	10·718	= 0·001	−0·430	0·120
Injection (type 2)	8·462	< 0·01	−0·525	0·155
Injection (type 3)	0·96	—	−0·058	0·377

Second order comparisons:

Injection (type 1): Injection (type 2) 1·808 = 0·15.
Injection (type 1): Injection (type 3) 6·098 = 0·02.
Injection (type 2): Injection (type 3) 9·672 < 0·01.

TABLE 7. CORRELATION BETWEEN EFFECT ON OVARIAN WEIGHT AND EFFECT ON ATTAINMENT OF FEMALE STATE

Source of variation	No. of animals	Difference of mean ovarian weights (x)	'Q' (from Table 6)	$p = \frac{Q+1}{2}$	Expected probit (y)
Injection (type 1)	200	4·9367	−0·430	0·2853	4·432
Injection (type 2)	106	2·5979	−0·525	0·2375	4·287
Injection (type 3)	121	9·3511	−0·058	0·4708	4·927
Operation	427	14·9577	+0·412	0·7060	5·542

After appropriate weighting of the data the regression equation is $y = 3·95+0·106x$ with variance of gradient 0·000 084 67, so that gradient = 0·106 ± 0·0092; hence $t_{[400]} = 11·538$ and $P < 0·001$. Columns 3 and 6 and the regression equation are illustrated graphically in Fig. 1.

$$y = 3·95+0·106x.$$

The regression coefficient was 0·106 ± 0·0092, whence $t_{[400]} = 11·583$ and $P < 0·001$. The equation and the four points upon which it is based are illustrated graphically in Fig. 1. It will be seen that the regression of the probit of Q upon difference in mean ovarian weight is highly significant; that is to say there is an extremely close correlation between the effects of the various treatments upon sex reversal and the effects of these same treatments upon ovarian growth.

OVARIAN SIZE AT THE MOMENT OF ATTAINMENT OF A FUNCTIONAL FEMALE CONDITION

In all groups it was found that those animals, regardless of body weight, which had the largest ovaries had become females, while those with the smallest ovaries had remained in the non-sexual condition; there was a very narrow zone of overlap, i.e. the animal with the smallest ovary of those which had become females, had, in some groups, an ovary just a little smaller than the largest of those which remained non-sexual. Fig. 2 illustrates the range of ovarian weights in the various groups of animals referred to in the previous sections. Statistical analysis confirms visual impressions that there is a very strong correlation between a large ovary and attainment of the female condition. The correlation coefficient is 0·99. Inspection of the graph also suggests that there is little variation in the ovarian weight at the boundary between those which had and those which had not become females. In all groups the largest ovary of a prawn which had remained non-sexual and the smallest ovary of a prawn which had become female were approximately 20 mg. Once more statistical analysis confirms this impression. Curve-fitting techniques applied to this whole assembly of ovarian weights and to the individual

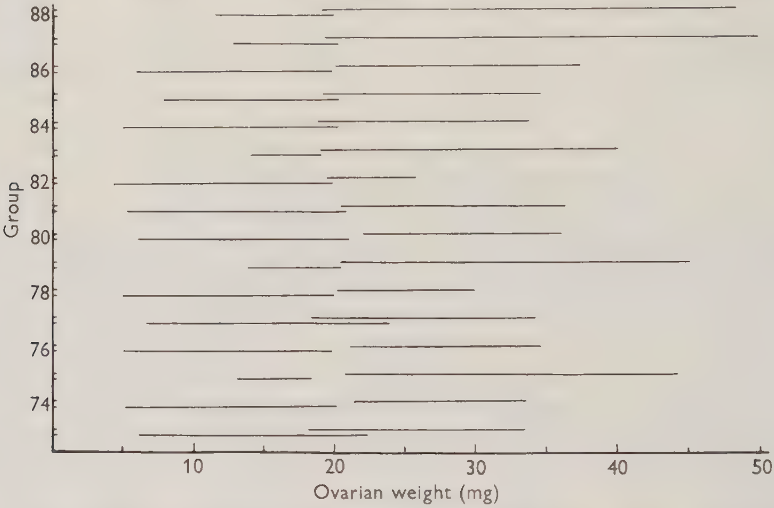


Fig. 2. The range of ovarian weights in animals of groups 73–88 (see Table 5). The lower line of each pair represents the range of weights found in those animals which remained non-sexual after the moult following experimental interference; the upper line the range in those which became functional females.

groups reveal insignificant departures from expectation with a $\chi^2_{[15]}$ of 11·85, whence $P = 0{\cdot}7$. In other words the ovarian weight at the moment of attainment of a functional female state was not modified by any of the treatments which I had applied.

DISCUSSION

These experiments make it evident that ovarian growth and maturation in *Pandalus borealis*, as in *Palaemon*, *Lysmata*, *Carcinus* and other species of decapods, is regulated, at least in part, by an ovary-inhibiting hormone produced by the X organ-sinus gland complex. This hormone is found in the medulla terminalis ganglionic X organ and in the sinus gland, but has not been found in the sensory pore X organ. It seems probable, therefore, that it is produced in the *MTGX*, stored in the sinus gland and released into the blood from there. No other tissue in the eyestalk which has been investigated had any effect upon ovarian growth or maturation. This is to be contrasted with the conclusions of Aoto & Nishida (1956) who, working with *Pandalus kessleri*, likewise found that eyestalk removal led to premature ovarian growth (at least during early summer). On histological grounds, however, they concluded that an ovary-inhibiting hormone was produced by the X organ (by which term they appear to mean the sensory pore X organ), while an antagonistic hormone was produced by the 'circum-orbital gland'. In *P. borealis* I have been able to find no trace of any hormone affecting gonadal or sexual development in the sensory pore X organ. Certainly there is a seasonal cycle in the histological appearance of this organ (Carlisle, 1959*a*), and since breeding is also seasonal, these two cycles must perforce be correlated. This is not, however, to be taken as a causal relationship without any other supporting evidence, and a diligent search has failed to produce any such evidence. We must conclude then that the seasonal changes in the *SPX* do not correspond to seasonal secretion of an ovary-inhibiting hormone, but rather are an expression of some other seasonal secretion, or annual cycle of metabolism. The same arguments apply also to the retroretinal tissue, which likewise undergoes a seasonal cycle in histological appearance. Here too I have failed to find any evidence of the secretion of hormones regulating any aspect of the sexual cycle. I believe that this tissue is probably homologous with that described as the circum-orbital gland by Aoto & Nishida. I have been able to find no evidence for any eyestalk hormone controlling any aspect of the sexual cycle of *P. borealis*, except the ovary-inhibiting hormone secreted by the neurosecretory cells of the *MTGX* and released into the blood by the neurohaemal endings of the sinus gland.

Similarly I could find no evidence that any hormone except one emanating from the *MTGX*-sinus gland complex, played any part in governing the change from the non-sexual to the female phase. Extracts of the *SPX*, of the retroretinal tissue or of the medulla externa were without effect, while eyestalk removal increased the proportion which became females at the next moult, an effect which was countered by the injection of extracts of sinus gland or of *MTGX*. We must conclude that some hormone produced in the *MTGX* and released into the blood at the sinus gland is responsible for restraining the attainment of the functional female condition. It is possible, but unlikely, that the vas deferens gland plays some part in this; the point requires further investigation. I have no evidence as to any part played by the Y organ.

The high degree of correlation found between the effects of the various treatments upon the attainment of the female form and upon ovarian inhibition makes it seem probable that but one hormone is concerned in inhibiting premature ovarian growth and in restraining the change from the non-sexual to the female condition. At the moment of this change the ovary seems always to have reached a certain minimum size. This seems unlikely to be a causal relationship but rather an expression of the same agent acting upon both the change of status and upon ovarian development.

I should like to acknowledge the friendly assistance afforded to me by the former director, Dr G. Gustafson, of the K. Svenska Vetenskapsakadamien Kristinebergs Zoologiska Station, and to thank him for all he has done to make three visits to the laboratory pleasurable as well as profitable.

SUMMARY

Ovarian growth and vitellogenesis are inhibited by the ovary-inhibiting hormone secreted by the medulla terminalis ganglionic X organ and the sinus gland. No other tissue within the eyestalk has been found to have any action upon ovarian growth. Similarly the change from the non-sexual condition to the functional female state is inhibited by a hormone emanating from the same centres, while no other tissue within the eyestalk has been found to have any effect. The two inhibitions show a high degree of correlation which makes it probable that they represent two responses to the same hormone.

REFERENCES

Allen, J. A., 1959. On the biology of *Pandalus borealis* Krøyer, with reference to a population off the Northumberland coast. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 189–220.

Aoto, T., 1952. Sexual phases in the prawn, *Pandalus kessleri* Czerniavski, with special reference to the reversal of sex. *J. Fac. Sci. Hokkaido Univ.*, Ser. 6 (Zool.), Vol. 11, pp. 1–20.

Aoto, T. & Nishida, H., 1956. Effect of removal of the eyestalks on the growth and maturation of the oocytes in the hermaphrodite prawn, *Pandalus kessleri*. *J. Fac. Sci. Hokkaido Univ.*, Ser. 6 (Zool.), Vol. 12, pp. 412–24.

ARVY, L., ECHALIER, G. & GABE, M., 1954. Modifications de la gonade de *Carcinides* (*Carcinus*) *moenas* L. (Crustacé décapode), après ablation bilatérale de l'organe Y. *C.R. Acad. Sci., Paris*, T. 239, pp. 1853–5.

BALSS, H., 1930. Decapoda. In Kükenthal & Krumbach's *Handbuch der Zoologie*, Bd. 3, Hft. 1, pp. 840–1038.

BERKELEY, A., 1930. The post-embryonic development of the common pandalids of British Columbia. *Contr. Canad. Biol.*, N.S., Vol. 6, No. 6, 85 pp.

CARLISLE, D. B., 1953*a*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). V. The ovarian inhibiting hormone and the hormonal inhibition of sex-reversal. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 355–72.

—— 1953*b*. Note préliminaire sur la structure du système neurosécréteur du pédoncule oculaire de *Lysmata seticaudata* Risso (Crustacé). *C.R. Acad. Sci., Paris*, T. 236, pp. 2541–2.

—— 1953*c*. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). VI. Notes on the structure of the neurosecretory system of the eyestalk. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 435–47.

—— 1954. The X organ-sinus gland complex, somatotropin, the ovarian inhibiting hormone and sex-reversal in *Lysmata* (Natantia, Hippolytidae). *Pubbl. Staz. zool. Napoli*, Vol. 24, Suppl., pp. 79–80.

—— 1959*a*. On the sexual biology of *Pandalus borealis* (Crustacea Decapoda). I. Histology of incretory elements. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 381–94.

—— 1959*b*. On the sexual biology of *Pandalus borealis* (Crustacea Decapoda). II. The termination of the male phase. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 481–91.

—— 1959*c*. Sexual differentiation in Crustacea Malacostraea. *Mem. Soc. Endocrinol.*, No. 7, (in the Press).

CARLISLE, D. B. & DOHRN, P. F. R., 1952. Sulla presenza di un ormone d'accrescimento in un crostaceo decapode, la *Lysmata seticaudata* Risso. *Ric. Sci. Torino*, Vol. 23, pp. 96–100.

—— —— 1953. Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda). II. Experimental evidence for a growth and moult-accelerating factor obtainable from eyestalks. *Pubbl. Staz. zool. Napoli*, Vol. 24, pp. 69–83.

CARLISLE, D. B. & KNOWLES, F. G. W., 1953. Neurohaemal organs in crustaceans. *Nature, Lond.*, Vol. 172, pp. 404–5.

—— —— 1959. *Endocrine control in crustaceans.* 120 pp. Cambridge University Press.

CAROLI, E., 1917. *Miersia clavigera* Chun, stadia misidiforme di *Lysmata seticaudata* Risso. *Pubbl. Staz. zool. Napoli*, Vol. 2, pp. 177–89.

CHARNIAUX-COTTON, H., 1954. Découverte chez un Crustacé amphipode (*Orchestia gammarella*) d'une glande endocrine résponsable de la différentiation de caractères sexuels primaires et sécondaires mâles. *C.R. Acad. Sci., Paris*, T. 239, pp. 780–2.

DOHRN, P. F. R., 1950. Studi sulla *Lysmata seticaudata* Risso (Hyppolitidae). I. Le condizioni normali della sessualità in natura. *Pubbl. Staz. zool. Napoli*, Vol. 22, pp. 257–72.

DOHRN, P. F. R. & HOLTHUIS, L. B., 1950. *Lysmata nilita*, a new species of prawn (Crustacea Decapoda) from the western Mediterranean. *Pubbl. Staz. zool. Napoli*, Vol. 22, pp. 339–47.

FINNEY, D. J., 1952. *Statistical method in biological assay.* 661 pp. London.

JÄGERSTEN, G., 1936. Über die Geschlechtsverhältnisse und das Wachstum bei *Pandalus*. *Ark. Zool.*, Bd. 28A, N:r 20, 26 Ss.

MISTAKIDIS, M. N., 1957. The biology of *Pandalus montagui* Leach. *Fish. Invest., Lond.*, Ser. 2, Vol. 21, No. 4, 52 pp.

PANOUSE, J. B., 1943. Influence de l'ablation du pédoncule oculaire sur la croissance de l'ovaire chez la crevette *Leander serratus*. *C.R. Acad. Sci., Paris*, T. 217, pp. 553–5.

PANOUSE, J. B., 1944. L'action de la glande du sinus sur l'ovaire chez la crevette *Leander*. *C.R. Acad. Sci., Paris*, T. 218, pp. 293–4.

—— 1946. Recherches sur les phénomènes humoraux chez les Crustacés. L'adaptation chromatique et la croissance ovarienne chez la crevette *Leander serratus*. *Ann. Inst. océanogr., Monaco*, T. 23, pp. 65–147.

—— 1947. Les correlations humorales chez les Crustacés. *Ann. biol.*, T. 23, pp. 33–70.

—— 1948. La glande du sinus et la maturation des produits génitaux chez les crevettes. *Bull. biol.*, Suppl. 33, pp. 160–3.

PIKE, R. B., 1952. Notes on the biology of the prawn *Pandalus bonnieri* Caullery. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 259–67.

RASMUSSEN, B., 1953. On the geographical variation in growth and sexual development of the deep sea prawn. *Rep. norweg. Fish. Invest.*, Vol. 10, No. 3, 160 pp.

RUNNSTRÖM, S., 1925. Beitrag zur Kenntnis einiger hermaphroditischen, dekapoden Crustaceen. *Bergens Mus. Skr.*, N.F., Bd. 3, pp. 1–115.

SPITSCHAKOFF, T., 1912. *Lysmata seticaudata* Risso, als Beispiel eines echten Hermaphroditismus bei den Decapoden. *Z. wiss. Zool.*, Bd. 100, Ss. 190–209.

TAKEWAKI, K. & YAMAMOTO, Y., 1950*a*. Hormonal regulation of ovarian development in the shrimp, *Paratya compressa*. *Annot. zool. Jap.*, Vol. 23, pp. 187–90.

—— —— 1950*b*. Acceleration of ovarian growth following removal of eyestalks in the shrimp, *Paratya compressa*. *Zool. Mag. Tokyo*, Vol. 59, pp. 5–7.

YULE, G. U., 1900. On the association of attributes. *Phil. Trans.*, A, Vol. 194, pp. 257.

YULE, G. U. & KENDALL, M. G., 1945. *An introduction to the theory of statistics*. Edit. 13, 570 pp. London.

J. mar. biol. Ass. U.K. (1959) 38, 507–524
Printed in Great Britain

ON THE BIOLOGY OF THE OPISTHOBRANCH *PLEUROBRANCHUS MEMBRANACEUS*

By T. E. Thompson and D. J. Slinn
Marine Biological Station, Port Erin, Isle of Man

(Plate I and Text-figs. 1–8)

On 5 November 1958 our attention was drawn, by Mr A. K. Nagabhushanam, to the presence in Port Erin bay of large numbers of swimming gastropods. These gastropods proved on examination to be *Pleurobranchus membranaceus* (Montagu), a species generally believed to be exclusively benthic in habit. This paper is a report on this occurrence and on some aspects of the biology of *P. membranaceus*.

For 4 days these animals were abundant in the bay and hundreds could have been captured with a hand-net from the steps of the Raglan Pier. Only approximately forty specimens were taken, this number being ample for our purpose. Between 5 and 9 November, numbers were stranded on the beach by the receding tide. Although many local fishermen consented to look out for these easily recognizable animals, and the Port Erin research vessels were out each day, no record of their swimming outside the bay was obtained, although specimens were occasionally dredged. On 9 November they disappeared from the bay without trace, and otter trawl and D-net hauls in the bay yielded only two further specimens; none were found on frequent shore-collecting expeditions. However, on 2 December five more specimens were captured swimming near the Raglan Pier and occasional individuals were seen in the same place on 9 December. During the periods of their abundance it was ascertained that they swam during the hours of darkness as well as in daytime.

Hydrographic conditions were not unusual during this invasion; temperatures taken in Port Erin bay during November and December 1958 were only about 1° C above the grand monthly means (see Bowden, 1955), while the salinities of samples collected at a position 54° 05′ N., 4° 50′ W. (about 3 miles N. 65° W. of Port Erin and 3 miles N. 12° E. of Chicken Rock) were about 0·3‰ below the mean for the area.

A search of the literature brought to light only three references to swimming in *P. membranaceus*. Pruvot-Fol (1954) states that this species (referred to as *Oscanius tuberculatus*, pp. 220–2) 'nage avec des contorsions du pied', but gives no details. Garstang (1890, pp. 418–20) quotes observations made by A. R. Hunt on an invasion of Torbay by numbers of *P. membranaceus* in

December 1873 and January 1874; Hunt observed that the animal swims on its back, 'alternately flapping, with wave-like contractions from before backwards, the two halves of its broad foot. The mantle flaps assist also in the action.' Garstang (1890) also mentions that these animals can secrete a substance which will redden blue litmus and he states (without giving reasons) that this substance is sulphuric acid. Garstang (1892) mentions an invasion of Plymouth Sound in September 1892 by young *P. membranaceus*; numbers were seen on the night of 21 September swimming at the surface of the sea. Hartley (1940) states that Saltash was invaded by large numbers of pleurobranchids in October 1936, but he does not say whether these animals were swimming. Except that Yonge (1949, p. 252) mentions that *P. membranaceus* feeds on simple ascidians (also inferred by Hunt (1925)), this is all that is known of the natural history of the species.

Nomenclature of British animals referred to herein is according to the Plymouth Marine Fauna list (Marine Biological Association, 1957); in the case of foreign species, the name used by the author cited is employed.

Material for sectioning was fixed in Zenker's fluid (with or without acetic acid), cleared in amyl acetate (Barron, 1934) and embedded in Hance's rubber wax (Gurr). The stains employed were Heidenhain's alum haematoxylin, Mayer's haemalum and, as counterstains, eosin and alcian blue 8 GS (Steedman, 1950). Methods used in the investigation of the nature of the acid secretion will be described in context.

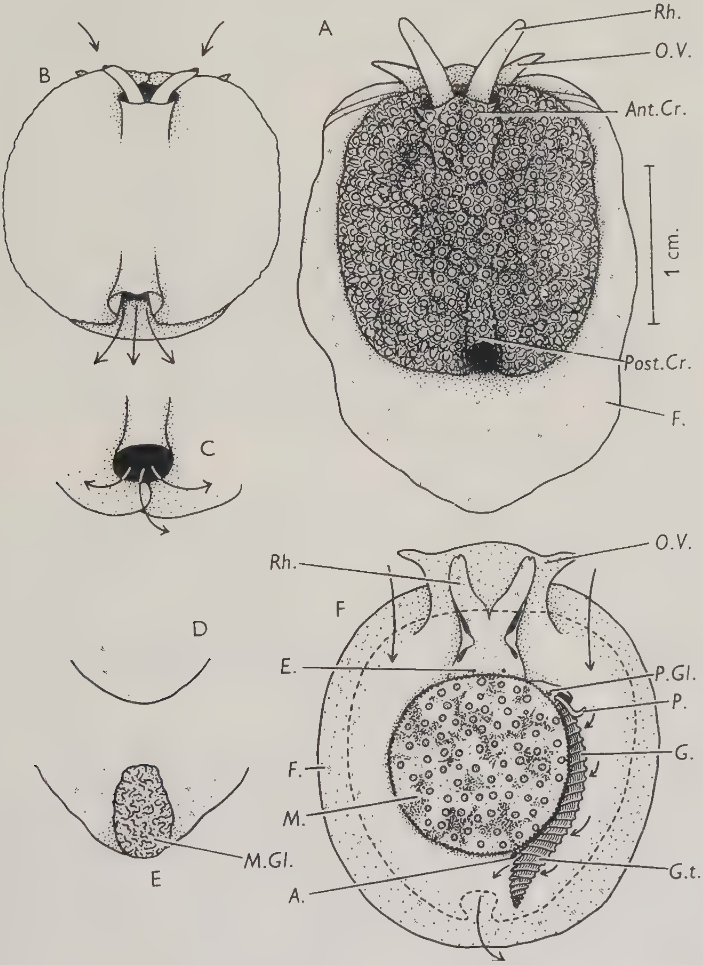
DESCRIPTION OF THE SPECIMENS

The external features and parts of the internal anatomy are shown in Text-figs. 1–6. Only those features which are not immediately apparent from these illustrations will be described. The description in the first instance will apply to animals which are creeping; the changes in form when swimming will be dealt with later.

External features

The lengths of the November specimens varied between $1\frac{1}{2}$ and $3\frac{1}{2}$ cm, measured while they were extended in creeping. Individuals of up to $4\frac{1}{2}$ cm in length were found in December. According to Pruvot-Fol (1954) adults may attain a length of 6 cm. In aquaria in the laboratory they spent by far the greater part of the time creeping and usually would swim only if disturbed.

Text-fig. 1 A shows a specimen in the act of creeping on the bottom of a glass vessel in the typical gastropodan fashion. Noteworthy features are the anterior and posterior crenations in the mantle, the former giving rise to a wide temporary sheath for the rhinophoreal tentacles (Text-fig. 1 A, *Ant.Cr.*), while the latter forms an exhalent aperture for the respiratory current (Text-fig. 1 A, *Post.Cr.*). In some individuals the posterior edges of the mantle skirt may temporarily meet below this exhalent aperture, rendering it functionally a circular orifice (Text-fig. 1 C). When the animal is at rest (Text-fig. 1 B), with the foot contracted so that it does not project laterally



Text-fig. 1. External features of *P. membranaceus*, all drawings to the same scale: A, dorsal aspect of November specimen while creeping; B, dorsal aspect of November specimen while at rest; C, as for B, but showing alternative condition of posterior crenation; D, ventral aspect of tip of metapodium of November specimen; E, ventral aspect of tip of metapodium of mature specimen of June, 1958; F, dorsal aspect of November specimen with mantle skirt dissected away; the interrupted line represents the normal extent of the mantle skirt; the cut edge of the mantle is represented by a jagged line. The arrows in all cases show the direction in which ciliary currents impelled fine carmine particles. *A.*, anus; *Ant.Cr.*, anterior mantle crenation; *E.*, eye; *F.*, foot; *G.*, gill; *G.t.*, free tip of gill; *M.*, mantle; *M.Gl.*, metapodial gland; *O.V.*, oral veil; *P.*, penis; *Post.Cr.*, posterior mantle crenation; *P.Gl.*, prebranchial gland opening; *Rh.*, rhinophore.

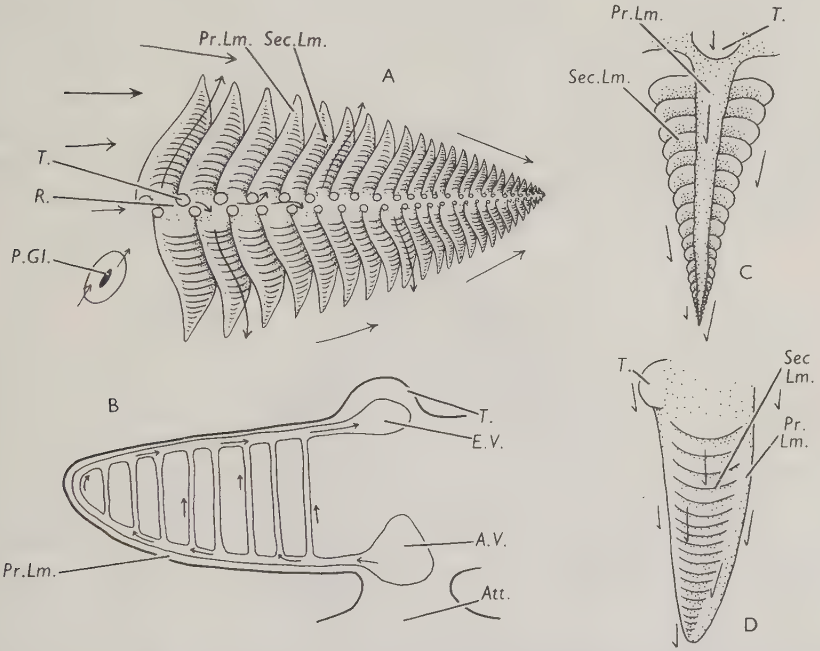
beyond the mantle skirt, this posterior crenation is relatively much wider than in the active animal. It is usually undetectable after death, which in part explains why it has not been described hitherto.

The dorsal surface of the mantle is covered with soft, conical, contractile tubercles. These tubercles are retracted if the animal is handled roughly; they are so contractile as to be almost undetectable in sections of the mantle. The basic colour of the mantle is pale brown, but patches of chocolate-coloured pigment are present between the tubercles. Slightly elevated ridges anastomose between the tubercles and the brown pigment is darkest alongside these. The pigment in sections can be seen to consist of large numbers of small spherical 'granules' lying against the basement membrane of the mantle epidermis. This epidermis is of single cell thickness and is remarkable for its apparent lack of histological differentiation. The cells are columnar and are greatly vacuolated so that they appear completely empty in sections. The only recognizable glands are scattered, small, unicellular mucus glands. There are no subepidermal glands in the mantle. Small aggregations of stellate calcareous spicules are present in the subepidermal layers of the mantle; these aggregations appear delicately pale blue-green in life and can be seen to be most abundant in the mantle skirt.

The foot is very mobile and varies greatly in its extent from one minute to the next. The peripheral regions may expand, becoming very thin, and approximately doubling the area of contact with the substratum when required (compare Text-figs. 1 A and 1 B). In life, a network of muscle fibres is visible through the foot epidermis. The basic colour of the foot is pale brown, but, as in the mantle, there are scattered patches of spicules and of dark brown pigment. It is particularly noticeable that, on the dorsal surface of the foot, only those regions not usually covered by the mantle skirt (i.e. the peripheral regions) bear pigment and spicules. Histologically the foot epidermis is very like the mantle. A feature hitherto regarded as diagnostic in earlier descriptions of the species, the metapodial gland, is absent in all the November specimens (see Text-fig. 1 D). That it is an organ developed at a later stage of life is confirmed by examination of preserved mature specimens (length 4 cm in formalin, captured in June 1958 by Mr A. K. Nagabhushanam) from the Port Erin area (see Text-fig. 1 E).

The rhinophores and oral tentacles bear patches of spicules and of brown pigment. The black-pigmented eyes (Text-fig. 1 F, *E.*), at the bases of the rhinophores, are hidden by the mantle when the animal is creeping.

The single gill is of the 'pectinate' type (Pelseneer, 1906) and is attached by its rachis to the lateral body wall on the right side along approximately three-quarters of its length. The free tip is muscular and mobile. The mantle skirt hides the gill from view when the animal is creeping. The respiratory currents set up by the cilia of the foot, mantle and gill lamellae are shown in Text-figs. 1 and 2. The respiratory chamber is elongated and tubular, bounded above by the mantle and below by the foot. It is open anteriorly, lateral to the right rhinophore, and posteriorly, at the temporary exhalent aperture formed by the mantle crenation (Text-fig. 1 A, *Post.Cr.*). Sections show the gill lamellae to bear large numbers of unicellular mucus glands, and the tubercles on the rachis (Text-fig. 2, *T.*) are rich in such glands, as well as being loaded with stellate spicules. Anterior to the gill is the opening of the pre-branchial gland (the poison gland of Bourne (1885) or excretory organ of Bojanus (Vayssière, 1898; Abbott, 1949)), whose functions are not understood. Near the posterior extremity of the gill is the anal opening (Text-fig. 1 F, *A.*). After feeding on *Ascidia* the faeces are very dark in colour and are expelled as elongated pellets, which, being on the 'downstream' side of the gill, present no problem in sanitation. Text-fig. 2 B shows the blood supply of a gill pinnule. The course of the flow of blood in the gill can be seen readily if a dilute suspension of indian ink is injected into the haemocoel.

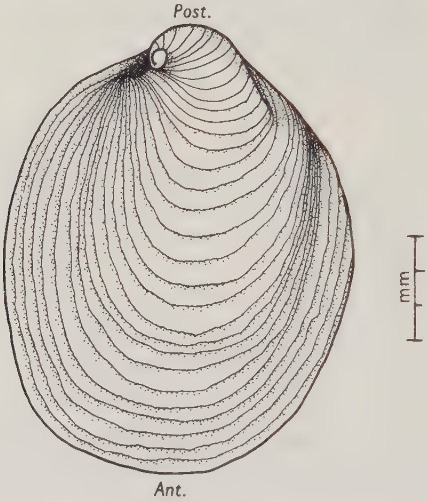


Text-fig. 2. The gill: A, gill dissected off and laid flat, the large arrow points towards the posterior of the animal; B, diagrammatic representation of the blood vessels in a gill pinnule, this is a simplified picture for there are in fact as many vertical vessels in the pinnule as there are secondary lamellae; C, single pinnule viewed from above; D, single pinnule viewed from the side. The arrows in A, C and D show the direction in which ciliary currents impelled fine carmine particles. In B they show the direction of the flow of blood in the branchial vessels. *Att.*, attachment of the gill rachis to the lateral body wall; *A.V.*, afferent vessel; *E.V.*, efferent vessel; *P.Gl.*, opening of prebranchial gland; *Pr.Lm.*, primary lamella; *R.*, rachis; *Sec.Lm.*, secondary lamella; *T.*, tubercle.

The ciliary currents over the rest of the body impel particles towards the posterior of the animal. No evidence of ciliary collection of food was obtained.

State of maturity

Microtome sections of the ovotestis of one of the larger (length 3½ cm) November specimens show that it was sexually immature. Spermatogenesis had reached an advanced stage, since tailed spermatozoa were visible in male follicles, but oogenesis was at a very early stage and the oocytes had not begun to accumulate cytoplasmic yolk. H. M. Lloyd (London Ph.D. thesis, 1952) has figured the anterior genital mass of *Pleurobranchus*, and her illustrations, together with dissections of mature specimens (collected by A. K. Nagabhushanam in June 1958) enabled comparison to be made with dissections of the sexual organs of our November specimens. This showed clearly that the genital mass of our specimens was in all respects in a rudimentary condition, although all the organs were present. The *external* genitalia, however, have all the appearance of maturity and the penis is long, and, in life, very mobile. This emphasizes the danger of judging a gastropod mature merely because the externally visible genital organs have the appearance of full development.



Text-fig. 3. The shell; dorsal aspect.

None of our specimens was observed to copulate or lay eggs either in the sea or in the laboratory.

FEEDING BEHAVIOUR

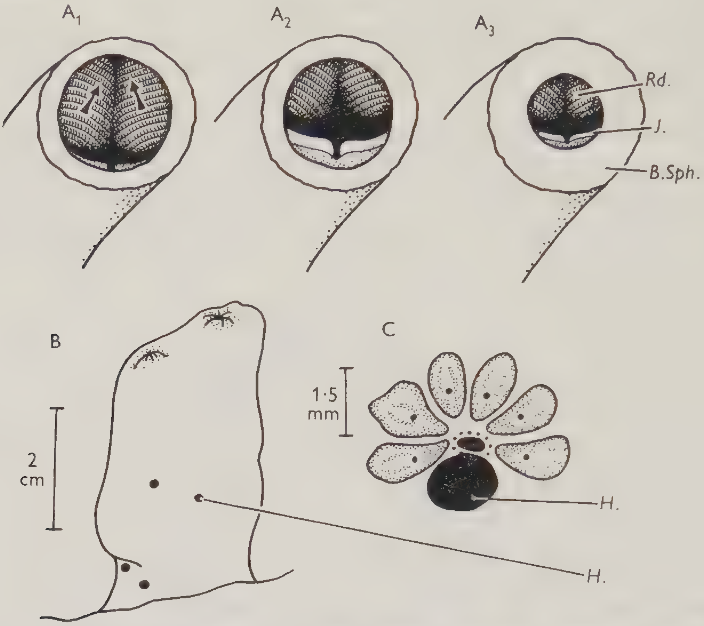
Specimens killed immediately after capture while swimming had no recognizable matter in the stomach. A little white flocculent matter of unknown nature was all that could be found. This white matter was certainly not composed of any of the usual planktonic organisms.

In the laboratory they fed readily on both compound and simple ascidians. Starved animals will, when creeping on the meniscus of the water, extrude the proboscis, and observations on such animals, together with those on specimens actually feeding, go to make up the ensuing description.

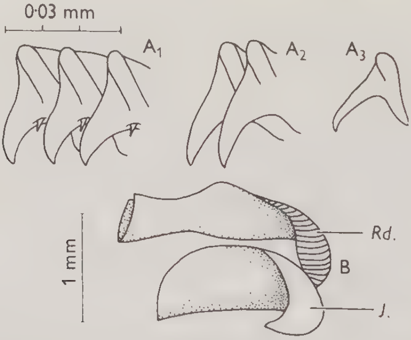
Yonge's (1949, p. 252) account of the patience of *P. membranaceus*, awaiting the relaxation of the siphons of the ascidian before shooting in the proboscis, could not be confirmed. *P. membranaceus*, like most opisthobranchs, is a voracious feeder, dealing with its prey with scant ceremony and considerable mechanical efficiency. No constant region of the prey is attacked.

Text-fig. 6A shows the alimentary canal dissected out entire. A conspicuous feature is the great development of the oral canal to form a long introvert. By turning the oral canal inside out, a highly mobile proboscis, up to a centimetre in length, is extruded through the mouth. Patches of brown pigment on the inside in the retracted state are external when the proboscis is extruded. The tip of the extended proboscis is formed by the muscular buccal sphincter (Text-figs. 4A and 6C, *B.Sph.*), through which the radula and jaws can be protruded. The buccal armature is shown in Text-fig. 5. Text-fig. 4A shows the phases of action through which the radula and jaws pass during rasping. During the first phase (Text-fig. 4A_1) the radula is expanded and bulged out through the buccal sphincter, whose aperture dilates. As it makes its effective stroke (in the direction indicated by arrows in Text-fig. 4A_1) and is withdrawn, the two halves of the radula are brought together. The effect is to carry particles caught on the radular teeth upwards and inwards. While the radula is halfway through its effective stroke, the extrusion of the jaws through the buccal sphincter marks the beginning of the second phase (Text-fig. 4A_2). The jaws then make a short upward stroke; this it is inferred pushes further matter on to the radular teeth and the radula is then withdrawn into the buccal mass, followed by the jaws. The third phase (Text-fig. 4A_3) is a brief resting period with the buccal sphincter contracted and the buccal armature withdrawn before the next cycle of action begins. Each cycle occupies between one and two seconds.

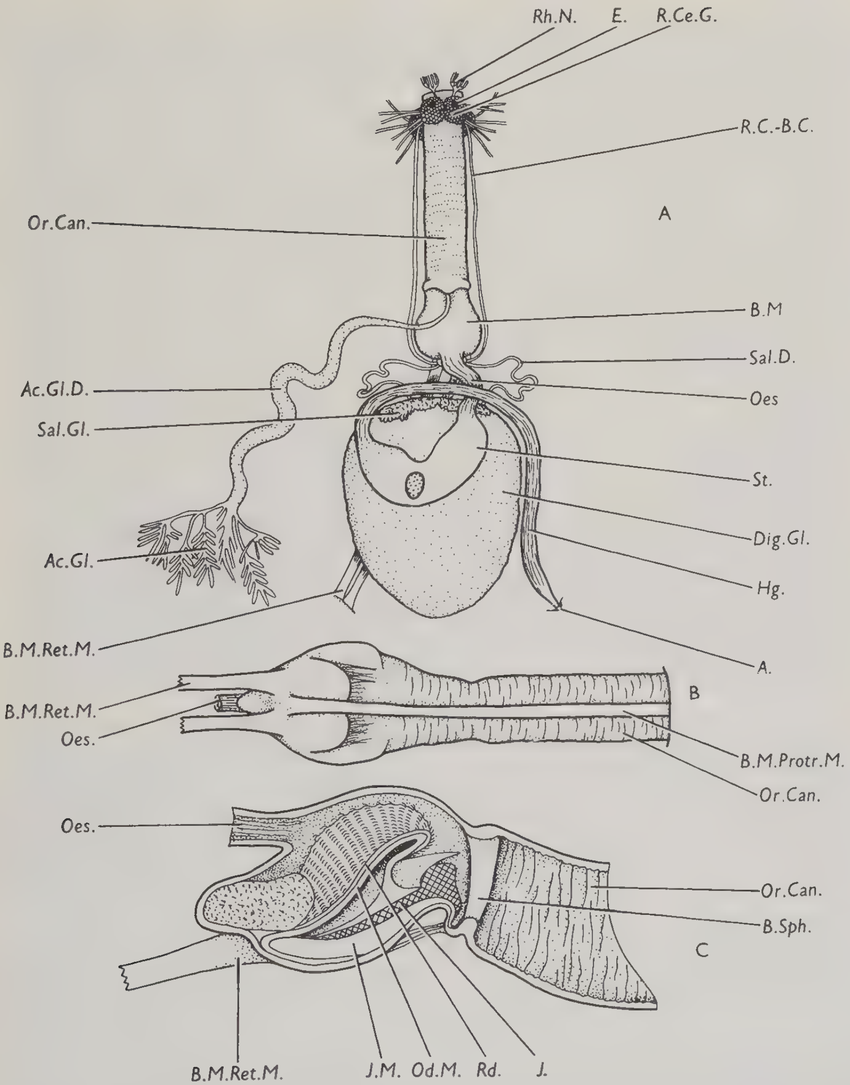
Extrusion of the proboscis is brought about by the action of the buccal mass protractor muscle (Text-fig. 6B, *B.M.Prot.M.*), aided by the extrinsic musculature of the buccal mass and also, no doubt, by pressure changes in the haemocoel. Its withdrawal is accomplished by a pair of stout buccal mass retractor muscles (Text-fig. 6A–C, *B.M.Ret.M.*), running from the buccal mass to the posterior body wall. Anatomical features associated with the great development of the oral canal are the relatively great length of the cerebro-buccal connectives (Text-fig. 6 A, *R.C.-B.C.*), of the salivary gland ducts (Text-fig. 6 A, *Sal.D.*) (the salivary glands themselves lying attached to the anterior face of the digestive gland) and the extreme extensibility of the oesophagus.



Text-fig. 4. The feeding mechanism: A, three phases of action of the radula and jaws at the tip of the extruded proboscis (see text for explanation); B, *Ascidia mentula* attacked by *Pleurobranchus*; C, *Botryllus schlosseri* attacked by *Pleurobranchus*. *B.Sph.*, buccal sphincter; *H.*, hole drilled by *Pleurobranchus*; *J.*, jaw; *Rd.*, radula.



Text-fig. 5. The buccal armature: A, radular teeth, showing teeth from centre of radula (A_1), more lateral teeth (A_2) and a tooth from the extreme edge (A_3); B, radula and jaws dissected out entire seen from lateral aspect, with anterior to the right of the page, dorsal to the top. *J.*, jaws; *Rd.*, radula.

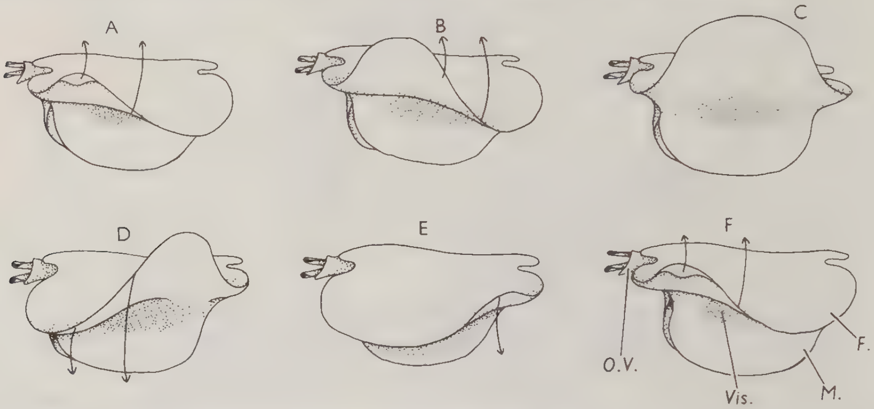

Rh.N.
E.
R.Ce.G.
R.C.-B.C.
A
Or.Can.
B.M
Sal.D.
Ac.Gl.D.
Oes
Sal.Gl.
St.
Dig.Gl.
Ac.Gl.
Hg.
B.M.Ret.M.
A.
B
B.M.Ret.M.
Oes.
B.M.Protr.M.
Or.Can.
Oes.
Or.Can.
B.Sph.
C
B.M.Ret.M.
J.M.
Od.M.
Rd.
J.

Text-fig. 6. The alimentary canal: A, alimentary canal dissected out entire from dorsal aspect with buccal mass retracted; B, ventral view of retracted buccal mass; C, left sagittal half of retracted buccal mass, cut surfaces left blank. *A.*, anus; *Ac.Gl.*, acid gland; *Ac.Gl.D.*, acid gland duct; *B.M.*, buccal mass; *B.M.Protr.M.*, buccal mass protractor muscle; *B.M. Ret.M.*, buccal mass retractor muscle; *Dig.Gl.*, digestive gland; *Hg.*, hindgut; *J.*, jaw; *J.M.*, jaw muscle; *Oes.*, oesophagus; *Od.M.*, odontophore muscle; *Or.Can.*, oral canal; *R.C.-B.C.*, right cerebro-buccal connective; *R.Ce.G.*, right cerebro-pleural ganglion; *Rd.*, radular membrane; *Rh.N.*, nerves to rhinophores; *Sal.D.*, duct of salivary gland; *Sal.Gl.*, salivary gland; *St.*, stomach.

Text-fig. 4B and C show the effect of the predation of *P. membranaceus* on two ascidians, *Botryllus schlosseri* and *Ascidia mentula*. Circular, straight-sided holes are drilled into the body of the prey.

SWIMMING BEHAVIOUR

Specimens observed in the sea were all swimming within a few centimetres of the surface; none was ever observed either rising towards or dropping from the surface. Animals brought back to the laboratory, however, spent by far the greater part of the time creeping on the bottom or sides of the glass aquaria in which they were kept. Violent agitation of the water in the vessel would usually elicit swimming behaviour on the part of at least some of the specimens.

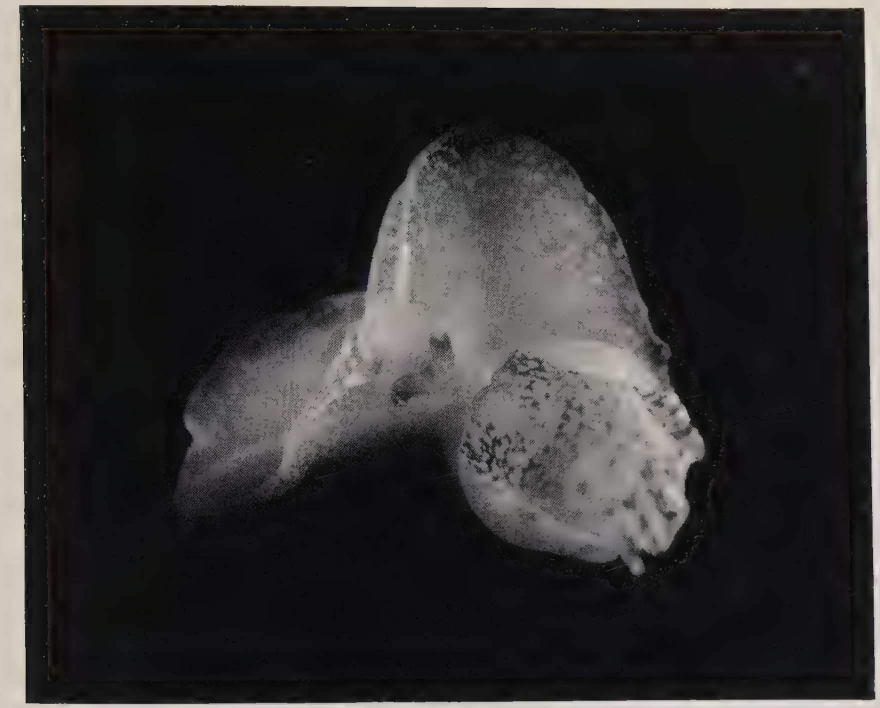


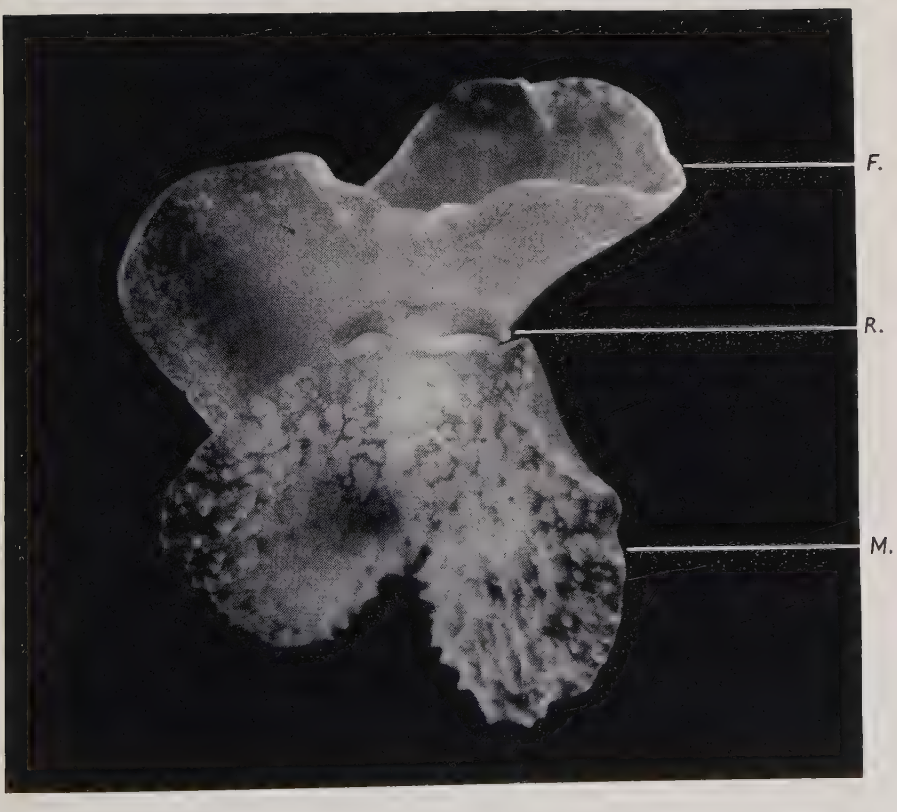
Text-fig. 7. The swimming mechanism of *Pleurobranchus*: the animals are shown as viewed from the morphological right side, but the gill is not illustrated. The swimming lobe of the left side is shown here, for simplicity, to be stationary in a relaxed position. For full explanation, see text. *F.*, foot; *M.*, mantle; *O.V.*, oral veil; *Vis.*, visceral mass, visible through the body wall.

Swimming is initiated by undulating movements of the epipodial lobes of the foot; these lobes become flattened, very thin, and greatly expanded compared with their normal (i.e. creeping) state. The animal turns over on to its back and may flutter over the bottom for some seconds before rising gently on a shallow climb to the surface. The sole propulsive organ in swimming, as in creeping, is the foot. The mantle is reflected away from the broad epipodial lobes; posteriorly the edges of the mantle skirt touch, forming a keel at the rear. The most striking feature of the swimming movements of the foot is the fact that the two lobes are not synchronous in action; that of one side under-

EXPLANATION OF PLATE I

Photographs taken through the side of a glass vessel of swimming *Pleurobranchus membranaceus*, viewed from the anterior; by electronic flash. ×2. *F.*, foot; *M*, mantle; *R.*, rhinophore.





(*Facing p.* 516)

goes its recovery stroke at the precise time when the other is in the phase of effective beat. This causes a high degree of instability in progression: the roll to either side is approximately 45° and so a movement through about 90° occurs between each pair of strokes. The anterior and posterior extremities of the foot are deeply notched (see Text-fig. 7), marking the line of discontinuity of action of the two lateral halves of the foot.

The cycle of activity through which each epipodial lobe passes may be best explained by reference to the figures. Text-fig. 7A shows the lobe at the commencement of the recovery stroke; this is initiated anteriorly, a wave of action passing back (Text-fig. 7B) until the whole lobe is poised above the animal (Text-fig. 7C). The effective stroke again begins anteriorly (Text-fig. 7D), passing backwards; the result is quite a powerful beat, providing a lifting component, with the thinner trailing margin of the lobe helping to provide a posterior component resulting in a small amount of forward progression (Text-fig. 7E). These swimming movements are not unlike those of a skate, except that, as stated above, the two swimming lobes act asynchronously in *Pleurobranchus*, and the wave of action in this gastropod is of relatively greater magnitude.

The mantle, reflected away from the body, provides no protection for the gill during swimming; this organ is widely exposed at each recovery stroke of the right epipodial lobe. This contrasts with the behaviour of the animal when creeping, with the gill completely enclosed from view in a furrow between the mantle skirt and the foot. While the animal is swimming the mantle acts as a double keel, counteracting to some extent the rolling and yawing consequent on the nature of the mode of progression.

Counts of from 55 to 60 strokes per minute (of each epipodial lobe) were made with animals in sea water at 15° C, in the laboratory.

While in the sea the animals did not appear to be swimming in any particular direction, and were completely at the mercy of the currents. *P. membranaceus* at this stage of its life cycle is more planktonic than pelagic.

ACID SECRETION

The opportunity was taken to re-investigate the statement of Garstang (1890) that *P. membranaceus* rendered itself distasteful to predators by the secretion of sulphuric acid. It is clear that Garstang was referring to previous work by some other naturalist, but we have been unable to trace the original source.

Preliminary tests were made by applying pH papers to the mantle and foot of a roughly stimulated animal. Blue litmus immediately turned red at the point of contact, and Johnson's pH papers indicated a pH in the region of 1. Repeated stimulation (with a glass rod) of a small area of the mantle soon brought about local exhaustion of the capacity to produce this acid. The secretion does not seem to be produced continuously, but only when the animal is abruptly disturbed; pH papers resting lightly on the mantle of a creeping specimen exhibited no colour change.

Some simple qualitative tests were then made in order to identify the anions present. To obtain samples of the acid secretion, the animal was passed rapidly through three changes of distilled water and excess water removed with filter paper. It was then placed in a Petri dish and the mantle stimulated with a glass rod. A few drops of distilled water were placed on it and then immediately drawn off with a fine pipette. Drops of this fluid were then tested for anions (see, for example, Belcher & Wilson, 1946). Chloride and sulphate were found to be present, but nitrite, nitrate and such organic radicals as oxalate, tartrate and citrate were not detected.

Gravimetric methods were used in an attempt to determine the sulphate: chloride ratio of the acid secretion, although the very small quantities of material available for testing rendered the results variable. Several animals were passed quickly through three changes of distilled water and then roughly stimulated with a glass rod. After stimulation the animals were washed in a minimum amount of distilled water, and determinations carried out on the combined washings (about 1–2 ml.).

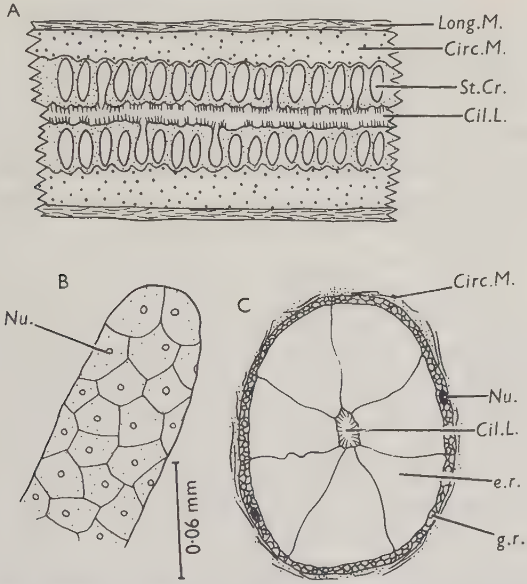
Tests for proteins, with such coagulating reagents as mercuric chloride, ethyl alcohol and concentrated nitric acid were negative. Therefore it was assumed that proteins were not present in sufficient quantities to interfere with the gravimetric determinations.

Sulphate and chloride were precipitated as barium sulphate and silver chloride respectively, according to the procedure recommended by Vogel (1951). To reduce the effects of co-precipitation of the sulphates of sodium and potassium, this precipitation was carried out in the presence of picric acid. Precipitates were separated from the fluid by means of a centrifuge. The results of four separate determinations varied rather widely, giving a mean $SO_4'':Cl'$ ratio of 0·75 ± 0·19 : 1. Two determinations carried out on local sea water using the same technique gave ratios of 0·13 : 1 and 0·14 : 1, compared with a mean value of 0·13991 : 1 found by Bather & Riley (1954) for the sulphate-chlorinity ratio of Irish Sea water. This fairly close agreement suggests that the technique used to obtain the fluid is the cause of the variation in our results, rather than the method of analysis.

Thus, when disturbed, *Pleurobranchus* produces an acid fluid of approximately pH 1, in which sulphate and chloride are the only anions present in appreciable amounts. The proportion of sulphate to chloride is much greater than in sea water. No analyses were made of the internal body fluids of *Pleurobranchus* due to the uncertainty of knowing whether a sample was contaminated with acid fluid from the epidermis or the acid gland. Robertson (1949) has given figures for the ionic composition of the body fluid of *P. membranaceus*, and he found the sulphate: chloride ratio to be about 0·143 : 1, only slightly above that of sea water.

Sites of production of the acid secretion

The general body surface. As mentioned above, the mantle and foot are both able to produce the acid fluid. The histological structure of the epidermis in *Pleurobranchus* is apparently simple and is quite unlike that of a dorid nudibranch (Thompson, 1958), in which large numbers of unicellular and multicellular epidermal and subepidermal glands open to the surface all over the mantle and its tubercles. In *Pleurobranchus* there are no subepidermal glands associated with the mantle or foot; there are only scattered small unicellular mucus glands of the usual type associated with ciliated epithelia.



Text-fig. 8. The acid gland: A, diagrammatic longitudinal section through the acid gland duct; B, branch of the acid gland, stained intra-vitally with methylene blue; C, transverse section through a branch of the acid gland, fixed Zenker-without-acetic, stained Heidenhain's alum haematoxylin. *Cil.L.*, ciliated central lumen; *Circ.M.*, circular muscle fibres; *e.r.*, empty region; *g.r.*, granular region; *Long.M.*, longitudinal muscle fibres; *Nu.*, nucleus; *St.Cr.*, storage crypts.

The acid gland (Text-fig. 6A, *Ac.Gl.*, and Text-fig. 8). This is a large, ramifying gland, communicating with the roof of the buccal mass by a stout duct. Tests performed on extracts of this gland showed that it produced a strongly acid fluid. Text-fig. 8B shows an ultimate branch of the gland and Text-fig. 8C a section through such a branch. The gland cells (see Text-fig. 8C) have the same appearance in sections as the cells of the mantle and foot epidermis. The main duct (Text-fig. 6A, *Ac.Gl.D.*, and Text-fig. 8A) has two muscle layers and huge numbers of crypts opening off the central ciliated lumen. It is inferred that the secretion of the gland cells is stored in this spongy duct, to be expelled rapidly when required, by contraction of the muscle layers.

To summarize: acid secretions are produced by the mantle and foot epidermis and by the ramifying median buccal gland (therefore called the 'acid gland'). The primary characteristic of the cells responsible for the secretions is a striking histological anonymity. They appear completely empty in sections; this, it is interesting to note, contrasts with the appearance of mammalian oxyntic cells, whose function is believed to be the secretion of hydrochloric acid (see Carleton & Leach, 1949).

Behaviour of would-be predators

None of the following carnivores would ingest *Pleurobranchus* in tests carried out at the Port Erin Aquarium: *Tealia felina*, *Blennius pholis*, *Pholis gunnellus*, *Solea solea*, *Pleuronectes platessa*, *Gadus calarias* and *G. virens*. In all cases the carnivore 'tasted' the pleurobranchid, but discarded it immediately, and, in the case of the fish, violently. In the field, shoals of coalfish (*Gadus virens*) and numbers of herring gulls were observed to pay no attention to swimming pleurobranchids.

Warning coloration

Cooke (1895) and others state that the coloration of *Pleurobranchus membranaceus* acts as a warning, an advertisement of its odious taste. However, while a *Pleurobranchus* held in the hand may appear to be conspicuous, this is certainly not the case when it is creeping on stones or weed; the coloration of the mantle is efficiently cryptic.

On the other hand, while the pleurobranchid is swimming, the movements of the foot attract human attention and must be similarly obvious to fish. Nevertheless, this is very far removed from the full concept of warning coloration. While creeping in its natural habitat, *Pleurobranchus* is effectively camouflaged; while swimming it does not advertise its presence by striking coloration, but neither does it make the least effort at concealment.

DISCUSSION

Swimming is a well-documented phenomenon in gastropods; it is widespread in the group and the possession of a swimming mechanism can be only warily accepted as evidence of close relationship. The means by which swimming is effected are as follows. (1) By lateral waves passing backwards along the whole body, e.g. the nudibranchs *Dendronotus giganteus* and *Melibe leonina* (Agersborg, 1922), *Scyllaea pelagica* (Collingwood, 1881), *Phyllirhoe* and *Cephalopyge* (Morton, 1958*b*). The waves pass down each side alternately, and the mechanism may be assisted either by the presence of an oar-like tail, as in *Lomanotus* (Trinchese, quoted by Colgan, 1908), or vertical undulations of the head, as in *Plocamopherus* (Lowe, quoted by Alder & Hancock, 1845–55). (2) By vertical waves passing backwards along the mantle skirt, e.g. some tropical dorid nudibranchs (personal communication from J. S. Colman). (3) By movements of expanded lobes of the foot, as in most swimming gastropods. The action may resemble (as Morton, 1958*a*, points out) in essence either sculling, e.g. *Clione* (Morton, 1958*a*), Heteropoda (Morton, 1958*b*), or rowing (or even 'flying'), in which the lateral halves of the foot may either beat synchronously, e.g. *Akera* (Morton & Holme, 1955), *Gastropteron* (Morton, 1958*b*), *Limacina* (Morton, 1954), or beat asynchronously, e.g. *Pleurobranchus membranaceus.*

The majority of these gastropods spend most of their lives swimming; this is plainly not the case in *Pleurobranchus*, which has only occasionally been seen swimming. Pelagic seasonal swarming is a familiar phenomenon in marine animals and it is usually associated with feeding, reproduction or dispersal. None of these fits the facts concerning *P. membranaceus*, for this species apparently does not feed while swimming, is sexually immature during the requisite phase and already has (in the shape of a pelagic veliger phase) a dispersal mechanism of the usual molluscan type. The only other suggestion which we can advance at present is that this is a phenomenon similar to the much-debated inshore migrations of nudibranchs, namely, a means of bringing about aggregation for reproduction at a much later date. However, since it is unlikely that drifting in the surface layers of the sea will bring about any such aggregation, and since the existence of migrations in nudibranchs is denied by several authorities (a summary has been given by M. C. Miller, Liverpool Ph. D. thesis, 1958), this hypothesis is of questionable value. The true explanation will only be obtained when more facts concerning the natural history of *P. membranaceus* are discovered.

This report would be incomplete without some comments regarding the systematic position of the pleurobranchids. It is known that in the nervous system, the reproductive system,[1] the alimentary canal (with its 'holohepatic' arrangement—see Text-fig. 6), and features of the development (compare the mode of formation of the adult mantle in the metamorphosing larvae of *Berthellina* (Gohar & Abul-Ela, 1957) and *Adalaria* (Thompson, 1958)), the pleurobranchids are closely related to the dorid nudibranchs. This was recognized by Thiele (1931) who placed the pleurobranchids in the suborder Notaspidea, which, together with the suborder Nudibranchia, made up his order Acoela. In support of this is the arrangement of the exhalent respiratory aperture, formed by a temporary crenation in the mantle skirt (see Text-fig. 1A, *Post.Cr.*), rendering the anus in *Pleurobranchus* in the same position *functionally* as that of the dorid nudibranchs. A simple change in the position of the gill would turn the pleurobranchid into a dorid. A homology of the dorid branchial circlet with the 'tectibranch' gill was first suggested by Evans (1914). In addition the way in which the mantle skirt encloses the basal parts of the rhinophoreal tentacles in *Pleurobranchus* (see Text-fig. 1A, *Ant.Cr.*) is strikingly similar to a developmental stage of the dorid *Adalaria* (Thompson, 1958); in *Adalaria* the anteriorly extending mantle fold during metamorphosis encircles and isolates the developing rhinophores. *Pleurobranchus* clearly shows an evolutionary stage in the production of the dorid rhinophore, which in the adult dorid appears to arise from the mantle.

---

[1] H. M. Lloyd, London Ph.D. thesis, 1952.

The authors are indebted to Mr J. S. Colman for critical reading of the manuscript and for the provision of laboratory facilities. The work was done while one of us (T. E. T.) was the holder of a Leverhulme Fellowship in the University of Liverpool. We are very grateful for field observations on *Pleurobranchus* to Mr L. Collister, Mr J. Faragher, Mr K. Woodworth, Mr P. J. Miller and Mr R. G. Hartnoll. Mr A. K. Nagabhushanam kindly provided trawled specimens for dissection.

Note added in proof

Specimens dredged and trawled during the spring and summer of 1959 were, through the kindness of Mr P. J. Miller and Mr R. G. Hartnoll, made available to us. Specimens of up to 7 cm long were collected during April and May and these deposited spawn masses in captivity. The ability to swim if abruptly disturbed is possessed even by the largest individuals. No specimens were found on the shore during 1959. None was dredged or trawled after the end of May; the inference is that *Pleurobranchus membranaceus* has an annual life cycle (like that of many dorid nudibranchs), the adults dying at the close of the breeding season.

SUMMARY

An account is given of an invasion of Port Erin bay by large numbers of swimming *Pleurobranchus membranaceus* (Montagu).

Descriptions are given of the external features of the specimens, the state of maturity, feeding behaviour and swimming behaviour.

The sites of production and the nature of the acid secretions are described.

Thiele's placing of the pleurobranchids close to the dorid nudibranchs is discussed and additional evidence to support this view is furnished.

REFERENCES

ABBOTT, R. T., 1949. A new Florida species of the tectibranch genus *Pleurobranchus*. *Nautilus*, Vol. 62, pp. 73–8.

AGERSBORG, H. P. K., 1922. Notes on the locomotion of the nudibranchiate mollusk *Dendronotus giganteus* O'Donoghue. *Biol. Bull., Woods Hole*, Vol. 42, pp. 257–66.

ALDER, J. A. & HANCOCK, A., 1845–55. *A Monograph of the British Nudibranchiate Mollusca.* London: Ray Society.

Barron, D. H., 1934. Amyl acetate: a useful solvent for embedding masses. *Anat. Rec.*, Vol. 59, pp. 1–3.

Bather, J. M. & Riley, J. P., 1954. The chemistry of the Irish Sea. Part 1. The sulphate-chlorinity ratio. *J. Cons. int. Explor. Mer*, Vol. 20, pp. 145–52.

Belcher, R. & Wilson, C. L., 1946. *Qualitative Inorganic Microanalysis*, 68 pp. London: Longmans.

Bourne, A. G., 1885. On the supposed communication of the vascular system with the exterior in *Pleurobranchus*. *Quart. J. micr. Sci.*, Vol. 25, pp. 429–32.

Bowden, K. F., 1955. Physical oceanography of the Irish Sea. *Fish. Invest., Lond.*, Ser. 2, Vol. 18, No. 8, 67 pp.

Carleton, H. M. & Leach, E. H., 1949. *Schafer's Essentials of Histology*, 15th ed. 655 pp. London: Longmans.

Colgan, N., 1908. Contributions towards a revision of the genus *Lomanotus*. *Ann. Mag. nat. Hist.*, Vol. 2, pp. 205, 392.

Collingwood, C., 1881. On some new species of nudibranchiate Mollusca from the eastern seas. *Trans. Linn. Soc. Lond. Zool.*, Vol. 2, pp. 123–40.

Cooke, A. H., 1895. Molluscs. In *Cambridge Natural History: Molluscs and Brachiopods*, 535 pp. London: Macmillan.

Evans, T. J., 1914. The anatomy of a new species of *Bathydoris*, and the affinities of the genus: Scottish national antarctic expedition. *Trans. Roy. Soc. Edinb.*, Vol. 50, pp. 191–209.

Garstang, W., 1890. A complete list of the opisthobranchiate Mollusca found at Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 1, pp. 399–457.

—— 1892. Notes on the marine invertebrate fauna of Plymouth for 1892. *J. mar. biol. Ass. U.K.*, Vol. 2, pp. 333–9.

Gohar, H. A. F. & Abul-Ela, L. A., 1957. The development of *Berthellina citrina* (Mollusca, Opisthobranchiata). *Publ. mar. biol. Stat. Al Ghardaqa*, No. 9, pp. 69–84.

Hartley, P. H. T., 1940. The Saltash tuck-net fishery and the ecology of some estuarine fishes. *J. mar. biol. Ass. U.K.*, Vol. 24, pp. 1–68.

Hunt, O. D., 1925. The food of the bottom fauna of the Plymouth fishing grounds. *J. mar. biol. Ass. U.K.*, Vol. 13, pp. 560–99.

Marine Biological Association, 1957. *Plymouth Marine Fauna*, 3rd ed.

Morton, J. E., 1954. The biology of *Limacina retroversa*. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 297–312.

—— 1958*a*. Observations on the gymnosomatous pteropod *Clione limacina* (Phipps). *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 287–97.

—— 1958*b*. *Molluscs*, 232 pp. London: Hutchinson.

Morton, J. E. & Holme, N. A., 1955. The occurrence at Plymouth of the opisthobranch *Akera bullata*, with notes on its habits and relationships. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 101–12.

Pelseneer, P., 1906. Mollusca. Pt. v of *A Treatise on Zoology*, 355 pp. London: Black.

Pruvot-Fol, A., 1954. Mollusques Opisthobranches. *Faune Fr.*, No. 58, 460 pp. Paris: Lechevalier.

Robertson, J. D., 1949. Ionic regulation in some marine invertebrates. *J. exp. Biol.*, Vol. 26, pp. 182–200.

Steedman, H. F., 1950. Alcian blue 8GS: a new stain for mucin. *Quart. J. micr. Sci.*, Vol. 91, pp. 477–79.

Thiele, J., 1931. *Handbuch der systematischen Weichtierkunde*. Bd. 1. 778 pp. Jena: Fischer.

THOMPSON, T. E., 1958. The natural history, embryology, larval biology and post-larval development of *Adalaria proxima* (Alder & Hancock) (Gastropoda Opisthobranchia). *Phil. Trans.*, B, Vol. 242, pp. 1–58.

VAYSSIÈRE, A., 1898. Monographie de la famille des pleurobranchidés. I. *Ann. Sci. Nat. Zool.*, Sér. 8, Vol. 8, pp. 209–402.

VOGEL, A. I., 1951. *A Textbook of Quantitative Inorganic Analysis. Theory and Practice*, 2nd ed. 918 pp. London: Longmans.

YONGE, C. M., 1949. *The Sea Shore*, 311 pp. London: Collins.

J. mar. biol. Ass. U.K. (1959) 38, 525–529
Printed in Great Britain

A HOPPER FOR USE WHEN SIEVING BOTTOM SAMPLES AT SEA

By N. A. Holme
The Plymouth Laboratory

(Text-figs. 1 and 2)

When large quantities of bottom soil are brought up by the dredge or grab, the problem of sieving the material within a reasonable time presents itself. The usual practice is to wash the soil through a sieve or series of sieves, the finest being of the order of 1–2 mm aperture, according to the nature of the soil or the size of the animals to be collected. It is usually necessary to agitate the soil either by spraying with a hose or by shaking the sieve in a bath of water, in order that finer particles may pass through the sieve. Many of the more delicate animals are damaged by these methods, and Spooner & Moore (1940), working on the Tamar estuary, found it necessary to mix the mud carefully by hand with water to make a 'soup' before it could be sieved without damage to the fauna. For work at sea Hartman (1955) describes a machine by which sieving was accomplished with the aid of water sprays and mechanical agitation of the sieves with power provided by an electric motor.

The apparatus to be described provides a semi-automatic means of washing soil into suspension in sea water, the resulting suspension passing fairly readily through a sieve. The animals are little damaged by this method, as once the soil has been placed in the hopper it remains undisturbed until washed over into the sieve. The method is probably no faster than others, but the physical labour of sieving is almost eliminated and the apparatus can be left to run unattended while further samples are being taken. The apparatus may be used on any ship having an adequate water supply to the deck.

It consists essentially of a square wooden hopper, some 18 in. (46 cm) across, into which the whole sample of soil is tipped, standing on a sloping V-shaped wooden base. The base is supported on four legs, to such a height that a sieve can be placed beneath the spout at its lower end. The two halves of the base are each inclined at about 23° to the horizontal, while along its axis the base slopes down towards the spout at an angle of 10°. The base has low walls at its sides which stop the water spilling out as the ship rolls. There is a gap of 4 cm between the base and three sides of the hopper, around which water from the overhead jets flows, slowly, eroding the outside of the pile. At the lower side of the hopper is a rising 'gate' which is lowered as necessary to retain the sample when the hopper is being filled.

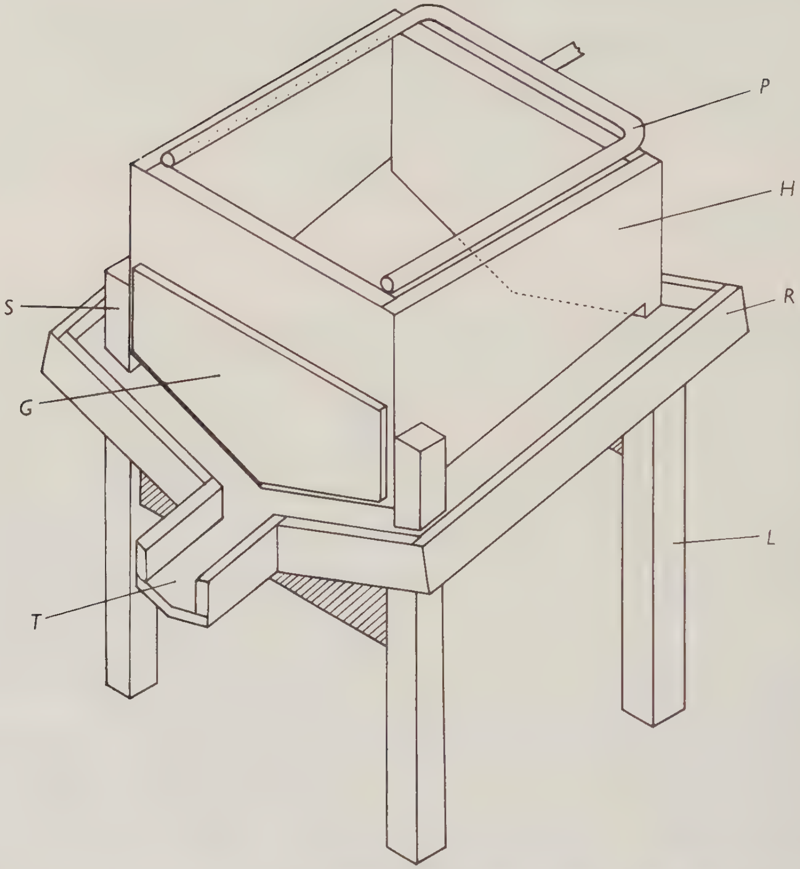
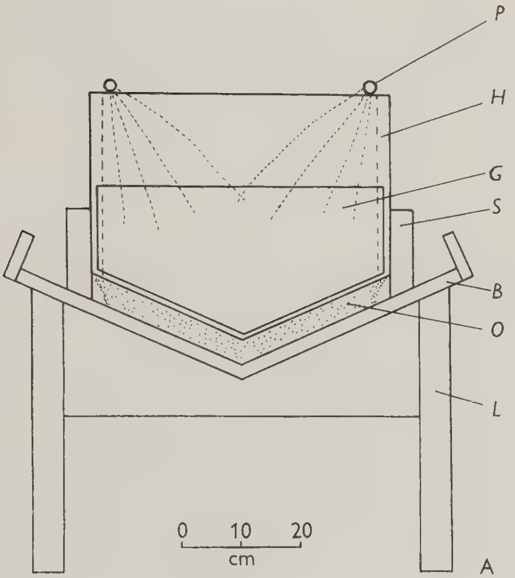


Fig. 1. General view of the hopper (isometric projection). Some constructional details are omitted. *P*, pipe supplying the jets along the top of the hopper; *H*, side wall of the hopper; *R*, retaining wall at side of base; *T*, spout; *G*, rising gate; *S*, short legs supporting lower side of hopper clear of base; *L*, legs supporting base.

The soil is broken down by jets of water issuing from rows of small holes in two lengths of pipe running along the top of two sides of the hopper. One inch bore polythene pipe, sealed at the outer end, has been used, and by using Yorkshire 'plastronga' elbow joints it is possible to vary the angle of the jets as required. The jets are arranged to fall at different angles to cover the surface of the soil in the hopper as completely as possible. Water accumulating on the surface excavates for itself cavities at the side of the heap, so draining down to the bottom of the hopper. Thence it travels around the base of the pile, out through the spout and into the sieve. It has not been found necessary to make an overflow near the top of the hopper, as the water always drains


P
H
G
S
B
O
L
0 10 20
cm
A

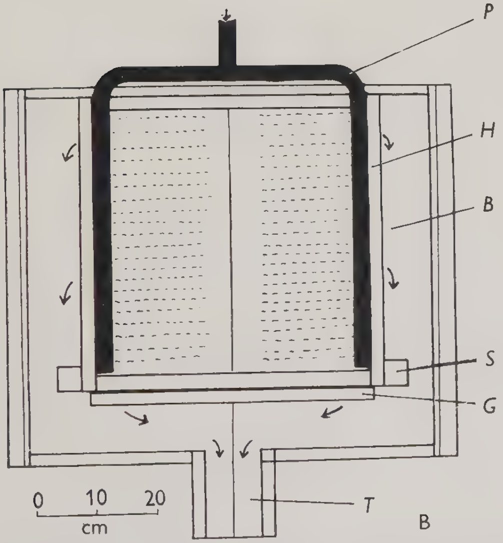

P
H
B
S
G
T
0 10 20
cm
B

Fig. 2. A, Transverse section of the hopper just above the spout. *B*, base; *O*, soil in hopper, showing eroding outer edge of the pile. Other lettering as in Fig. 1. B, Top view of the hopper.

away satisfactorily. Small animals and all fine material are washed over into the sieve; large animals and stones remain in the hopper, from which they may have to be removed from time to time if they cause obstruction to the flow of water.

Although the hopper may be left to run on its own for short periods, washing is speeded if the surface of the soil is occasionally stirred with a trowel. Lumps of mud are nearly always broken up before reaching the sieve, but where there is much shell or gravel the sieve becomes blocked from time to time. If this happens the sieve is thoroughly washed in a bath of water and the contents emptied out before continuing any further sieving.

Washing is little affected by the ship's motion, and the hopper can be used in any weather in which dredging or grab sampling is likely to be undertaken.

The use of a nest of sieves of decreasing mesh size is unsatisfactory with this hopper, since one or other of the sieves will soon choke and overflow if left unattended. The sieves used have a rectangular wood frame 45 × 35 × 20 cm deep (a sieve of larger area would no doubt be less often clogged, but this size was chosen as convenient for rinsing the sieve in a bath of water). The sieve has a handle at each end, and short legs at the corners to raise the gauze screen clear of the deck. A sieve in which the gauze is curved into an arc of a circle is less easily blocked than a flat one, and the sieves are provided with a side-window of gauze to prevent overflowing (cf. Spooner & Moore, 1940). A square or rectangular sieve is easier to empty than a round one, as the sievings can be washed into a corner and then rinsed into a jar with a little water.

I am indebted to Mr A. N. Bennett and to Mr F. G. C. Ryder and his workshop staff for constructing this apparatus, and to Mr P. G. Corbin for his helpful criticism of the manuscript.

SUMMARY

A hopper is described which facilitates the sieving of large quantities of bottom soil at sea. It can be left unattended for short periods, and considerably reduces the labour involved in sieving. Animals are probably less damaged by this than by the usual methods, as there is considerably less disturbance in the sieve. The apparatus may be used on any ship where there is an adequate water supply to the deck, and it can be operated successfully in moderately rough weather.

REFERENCES

Hartman, O., 1955. Quantitative survey of the benthos of San Pedro Basin, Southern California. Part I. Preliminary results. *Allan Hancock Pacific Expeditions*, Vol. 19, No. 1, 185 pp.

Spooner, G. M. & Moore, H. B., 1940. The ecology of the Tamar estuary. VI. An account of the macrofauna of the intertidal muds. *J. mar. biol. Ass., U.K.*, Vol. 24, pp. 283–330.

J. mar. biol. Ass. U.K. (1959) 38, 529–541
Printed in Great Britain

WIND CURRENTS, TIDAL STREAMS AND PLANKTON OFF THE NORTHUMBERLAND COAST

By Frank Evans
The Dove Marine Laboratory, Cullercoats*

(Text-figs. 1–7)

Investigations of the water movements off the southern Northumberland coast have until now been confined to the surface region. While important for navigational and similar purposes such investigations can give us no more than an indication of the total water flow along the coast nor can they elucidate the effects of currents on planktonic organisms which spend part or all of their existence away from the surface. It is for the latter reason that the series of investigations of sea surface currents made aboard the R.V. 'Alexander Meek' in 1956–7 (Evans, 1957) has now been supplemented by further series at two deeper levels.

The work of measurement was again undertaken on the stretch of coast between the River Tyne entrance (55° 01′ N., 1° 24′ W.) and Newbiggin Point (55° 11′ N., 1° 30′ W.) at a distance of about a mile from the shore. There now exist for this area records of (aperiodic) wind currents and tidal streams for 2 fathoms below the surface, for 6 fathoms below the surface and for approximately 3 fathoms off the bottom. In the work to be described the method used in measuring water flow followed that of the 1956–7 series; the track of a large, freely drifting drogue and buoy was plotted over half a lunar day. This was done to isolate the wind current from the general flow, observations beginning at various states of tidal stream and continuing for between 12 and 13 h, the exact period to be spent in tracking the buoy being determined from an inspection of the tide tables.

A description of the form of drogue and buoy used has been given in my earlier account; briefly, each drogue was an open-ended box 4 ft 6 in. high, of wood and canvas, cross-stayed with steel bar and attached by 1 in. circumference wire to a fisherman's dan buoy. Using this equipment runs at 2 and 6 fathoms were made by interposing the appropriate length of wire between the buoy and the suspended drogue. Runs with the drogue riding approximately 3 fathoms off the bottom were accomplished in the following way: a wire 15 fathoms long was used, this being 3 fathoms less than the extreme depth of water in which the gear was worked. The wire was shackled to both buoy and drogue, and close beneath the buoy a bight was taken out of the wire and stopped off with a bulldog grip (Figs. 1 & 2). The effective length of wire between the buoy and the drogue could be increased or decreased by slacking back the bulldog grip and adjusting the length of the bight. During the work soundings were taken on the ship's echo sounder and the length of wire in use varied accordingly.

---

* Present address: University College, Achimota, Ghana.

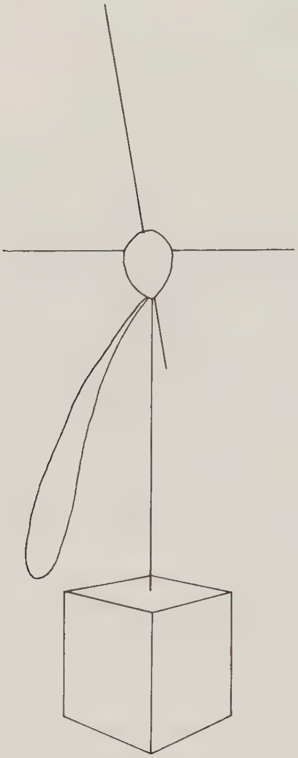


Fig. 1

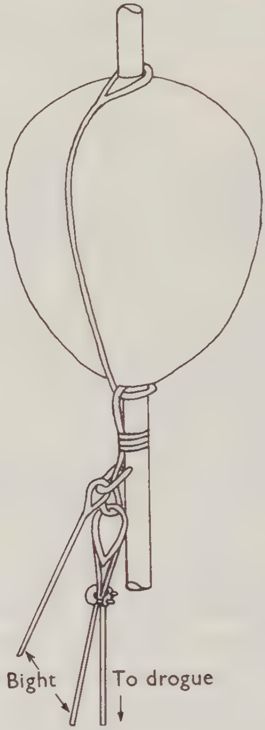


Fig. 2

Fig. 1. Attachment of the drogue to the buoy for runs near the bottom.

Fig. 2. Details of the attachment of the near-bottom drogue to the buoy.

Between January and July 1958, 5 runs were completed with a drogue riding at 2 fathoms' depth, 20 runs with a drogue riding at 6 fathoms' depth and 13 runs with a drogue riding about 3 fathoms off the bottom (Table 1). The results of the shallow water runs of 23 May and 4 July in which only a 6-fathom drogue was used have been utilized in both the 6-fathom records and those of 3 fathoms off the bottom. During the observations the mean depth of water was 11·2 fathoms (Table 2); consequently the 2-fathom drogue sampled near-surface currents, the 6-fathom drogue sampled mid-water currents and the drogue riding at 3 fathoms off the sea floor sampled near-bottom currents. For the sake of brevity, currents at these depths will therefore be referred to as near-surface, mid-water and near-bottom.

Drogue positions were fixed every 45 min by horizontal sextant angles of conspicuous marks ashore and, since the installation of a Decca Navigator in our ship, by an occasional Decca fix in thick weather. Wind speed and direction, measured by anemometer and compass, were noted at each fix, and a sounding was taken. Drogues were used in pairs, sampling two depths simultaneously. While this doubled the sampling rate over that of the 1956–7 series it possessed the disadvantage that after some hours the two drogues sometimes separated

TABLE 1. WIND CURRENT AND WIND OBSERVATIONS DURING THE YEAR

(Directions are true and measured from north throughout this account)

Date 1958	Set (°) Near surface	Set (°) Mid water	Set (°) Near bottom	Drift (miles) Near surface	Drift (miles) Mid water	Drift (miles) Near bottom	Downwind direction (°)	Downwind speed (knots)
29 Jan.	080	090	—	0·4	0·3	—	070	3·7
12 Feb.	135	358	—	1·4	0·5	—	097	13·5
20 Feb.	131	136	—	4·5	3·7	—	117	18·8
4 Mar.	112	128	—	3·8	2·1	—	090	17·4
11 Mar.	139	247	—	0·6	0·4	—	124	7·8
2 Apr.	—	169	164	—	0·5	0·9	253	6·0
21 Apr.	—	128	129	—	2·2	1·2	090	12·3
23 Apr.	—	357	340	—	0·7	0·6	018	12·0
29 Apr.	—	128	082	—	1·0	1·1	083	13·3
1 May	—	297	310	—	0·5	0·6	320	2·8
8 May	—	024	020	—	3·0	2·7	035	23·3
9 May	—	250	250	—	0·3	0·2	145	3·7
14 May	—	337	329	—	1·9	1·7	277	9·6
20 May	—	057	047	—	1·1	0·9	078	21·0
23 May	—	309	(309)	—	1·2	(1·2)	256	14·3
28 May	—	230	260	—	0·3	0·1	297	2·0
30 June	—	080	105	—	0·3	0·4	200	4·3
4 July	—	167	(167)	—	1·1	(1·1)	198	13·0
7 July	—	337	329	—	1·1	1·0	315	4·0
14 July	—	152	129	—	2·1	2·0	096	16·8

TABLE 2. MEAN DEPTH OF WATER DURING THE RUNS AND MEAN DEPTH OF THE NEAR-BOTTOM DROGUE

(Depths in fathoms)

Date 1958	Water depth	Drogue depth	Diff.	Date 1958	Water depth	Drogue depth	Diff.
29 Jan.	8·3	—	—	8 May	12·6	8·0	4·6
12 Feb.	12·8	—	—	9 May	9·8	7·5	2·3
20 Feb.	14·1	—	—	14 May	9·4	8·0	1·4
4 Mar.	14·7	—	—	20 May	12·9	9·5	3·4
11 Mar.	9·0	—	—	23 May	8·3	(6·0)	2·3
2 Apr.	8·7	7·0	1·7	28 May	9·6	8·0	1·6
21 Apr.	15·3	10·4	4·9	30 June	10·5	7·2	3·3
23 Apr.	11·7	9·6	2·1	4 July	7·6	(6·0)	1·6
29 Apr.	12·5	8·8	3·7	7 July	10·3	8·0	2·3
1 May	11·0	8·0	3·0	14 July	15·5	11·2	4·3
						Mean difference	2·8

so much that it was impossible to supervise them both from the ship and one had to be picked up and launched again close to the other, allowance for such relaunching being made in the plot of the buoy's track. At one time I had hoped to install a radio transmitter on one of the buoys whose signal could be received on the ship's direction finder but technical difficulties prevented this.

From the results obtained wind current and tidal stream data have been derived. The wind current was taken to be the residual current of each run, since over half a lunar day the tidal streams were held to have cancelled out. The set and drift of the wind currents together with accompanying wind speed are shown in Table 1. The five near-surface results confirm that surface water off the Northumberland coast moves in general into the quadrant to the right of downwind. This is not so at mid water nor near the bottom. Here currents flow as freely to the left of downwind as to the right and about one current in five has an upwind component. During the observations the mean vector downwind blew 092° at 4·9 knots or 61 miles per half lunar day, compared with 093° and 5·4 knots, or 67 miles per half lunar day during the 1956–7 observations. Direct comparison of current speed and direction between the series of 1956–7 and those of 1958 may therefore be made without undue error.

TABLE 3. DOWNWIND-CURRENT ANGLES DERIVED FROM TABLE 1

(Angles in degrees)

Date 1958	Near surface	Mid water	Near bottom	Date 1958	Mid water	Near bottom
29 Jan.	+10	+20	—	8 May	−11	−15
12 Feb.	+38	−99	—	9 May	+105	+105
20 Feb.	+14	+19	—	14 May	+60	+52
4 Mar.	+22	+38	—	20 May	−21	−31
11 Mar.	+15	+123	—	23 May	+53	(+53)
2 Apr.	—	−84	−89	28 May	−67	−37
21 Apr.	—	+38	+39	30 June	−120	−95
23 Apr.	—	−21	−38	4 July	−31	(−31)
29 Apr.	—	+45	−1	7 July	+22	+14
1 May	—	−23	−10	14 July	+56	+33

Mid-water average +5°

Near-bottom average −3°.

From Table 1 the mean vector current at mid water is 101° and 0·37 miles per half lunar day. Near the bottom it is 040° and 0·25 miles per half lunar day. This compares with 114° and 1·30 miles per half lunar day found near the surface in 1956–7. The mean vector current is thus reduced to 28% of the near-surface value at mid water and to 19% near the bottom. The average current, disregarding direction, is at mid water 1·22 miles per half lunar day and near the bottom 1·05 miles per half lunar day. These are 63% and 54%

respectively of the near-surface value of 1·95 miles per half lunar day recorded in 1956–7.

The proportional discrepancy between the mean vector currents and non-directional averages at the three levels is largely due to the greater directional randomness of the deeper currents. That it is not due to more random speeds of the deeper currents is shown by an examination of the simple expression *wind speed/current speed.* For the near-surface observations of 1956–7 we have an average value of 65·2, for mid-water 140·4 and for near the bottom 145·4. The coefficients of variability of these values are 56%, 51% and 49% respectively; i.e. randomness of wind current speed does not increase from surface to bottom.

From Table 3 the average of downwind-current angles for all observations works out to be, at mid-water +5° (current to the right of downwind) with standard deviation 63°, and near the bottom −3° with standard deviation 45°. During 1956–7 near-surface current deflexion was +40° with standard deviation 26°. Hence, away from the surface, deflection is less but deviation from the mean is greater. At neither mid water nor near the bottom is there a predominant current as defined in my earlier account (Evans, 1957, p. 496).

To a subscriber to Ekman's theory relating wind and ocean current direction these results are puzzling, for Ekman, it will be recalled, postulated that close to the surface a wind current would be deflected 45° to the right of downwind in the northern hemisphere and that the deflexion would increase with depth. The near-surface currents, but not the deeper currents, agree with this finding. None of the later modifications to Ekman's theory, including that of a wind blowing offshore across a land barrier (Proudman, 1953, p. 187) appear to remove the difficulty.

Current directions were tested against winds recorded during the 24 h preceding each observation, wind data being compiled from 3-hourly readings of an anemometer at Tynemouth. Agreement was poorer than with winds blowing at the time of observation. Current deflexion at mid-water was +19° with standard deviation 82° and near the bottom +6° with standard deviation 95°. It is clear that, as with near-surface water, the onset of a given wind and the appearance of the induced current are separated by only a short interval of time.

For comparison, the 9 a.m. winds observed at sea and at Tynemouth are shown in Tables 4 & 5. The winds encountered at sea form a good sample of the daily winds observed ashore and it is assumed that the wind currents found are likewise representative of conditions during the first 7 months of 1958.

The measurement of tidal streams was accomplished by subtracting from the plot of each day's run that portion which represented wind current. Of necessity it was assumed that the wind current flowed steadily and in a constant direction throughout each run. Then, by construction (Fig. 3), it was possible to find the points of slack water had there been no wind current. The tidal stream curve off Northumberland is approximately elliptical; therefore a line joining the two points of slack water is the major axis of the ellipse and represents the mean flow of both ebb and flood streams in magnitude and direction. Such tidal stream axes had a mean orientation of 164°–344° at all depths, maximum deviations being ±17°. Since the bearing of Newbiggin Point from the North Tyne Light is 343° the mean flow of tidal streams, unlike that of wind currents, is seen to be parallel to the coast.

TABLE 4. 9 A.M. WIND FORCES AT TYNEMOUTH AND AT SEA, EXPRESSED AS PERCENTAGE OF TOTAL OBSERVATIONS

	At Tynemouth	At sea
Observations	212	20
Calm	5·7	5
Force 1	10·8	5
Force 2	14·6	20
Force 3	25·0	25
Force 4	27·6	25
Force 5	9·9	5
Force 6	4·2	15
Force 7	0·5	0
Force 8	1·9	0

TABLE 5. 9 A.M. DOWNWIND DIRECTIONS AT TYNEMOUTH AND AT SEA, EXPRESSED AS PERCENTAGE OF TOTAL OBSERVATIONS

	At Tynemouth	At sea
Observations	212	20
Calm	5·4	5
000°–089°	24·0	15
090°–179°	37·3	45
180°–269°	14·6	25
270°–359°	18·4	10

The magnitude of the major axis of the tidal stream ellipse may appropriately be called the stream range, corresponding to the vertically measured tidal range. The stream range is found to vary rather less in proportion over the three levels than the wind currents. Near the surface 25 observations including those of 1956-7 yield an average range of 2·8 miles, at mid-water the range averages 2·7 miles and near the bottom, 2·5 miles.

Stream range varies under soli-lunar influence in the same manner as tidal range. Relationships have been established between stream range and predicted tidal range at the Tyne entrance. In Figs. 4–6 the stream range is compared with the mean tidal range for each day of observation. Regression lines are plotted for the following values: near the surface $t = 5{\cdot}3 + 1{\cdot}88s$; at mid-water $t = 4{\cdot}9 + 2{\cdot}29s$; and near the bottom $t = 6{\cdot}8 + 1{\cdot}79s$; where t is the predicted tidal range in feet and s the stream range in miles.

If the mean tidal range at the Tyne entrance is taken as 11 ft, then by Figs. 4–6 the mean stream range is 3·03 miles near the surface, 2·66 miles at mid-water and 2·35 miles near the bottom. Thus under average and parallel tidal conditions the stream range near the surface is about 0·4 miles greater than at mid-water and about 0·7 miles greater than near the bottom.

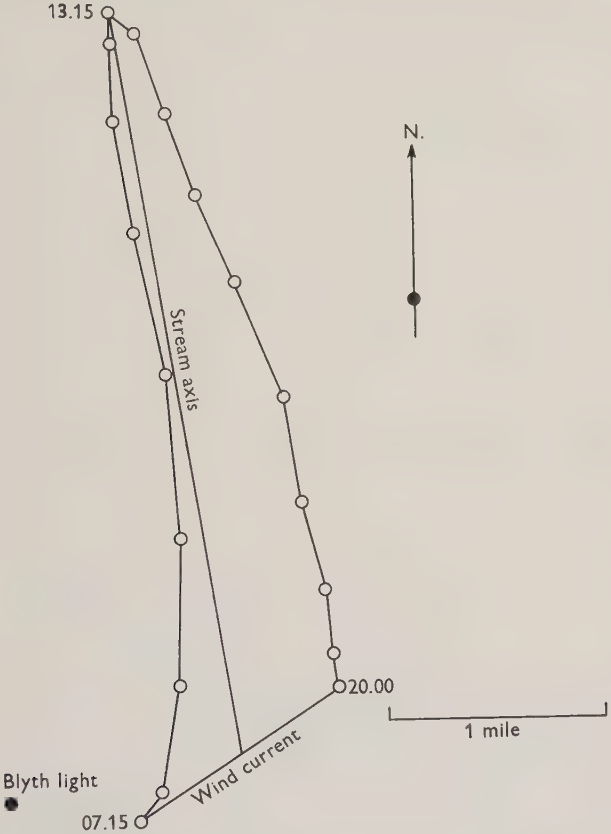


Fig. 3. Plot of the mid-water run of 20 May 1958 showing the extraction of wind current and stream range.

As a footnote to the stream range data the following may throw some light on the manner and time at which drifting material and the larvae of littoral animals are brought ashore on the Northumberland coast. (Until currents in and near the surf zone have been studied the final track before grounding must remain conjectural.) Near the surface the shape of the tidal stream ellipse is largely obscured by the wind current. The prevailing westerly wind combined with Coriolis force produces a predominant current moving southwards along the coast and somewhat offshore. Away from the surface,

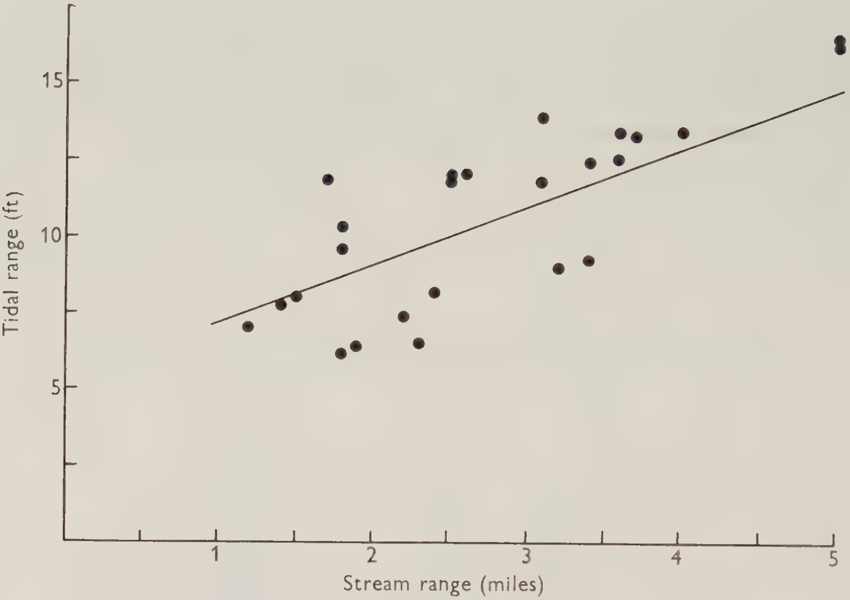


Fig. 4. Stream range compared with tidal range near the surface.

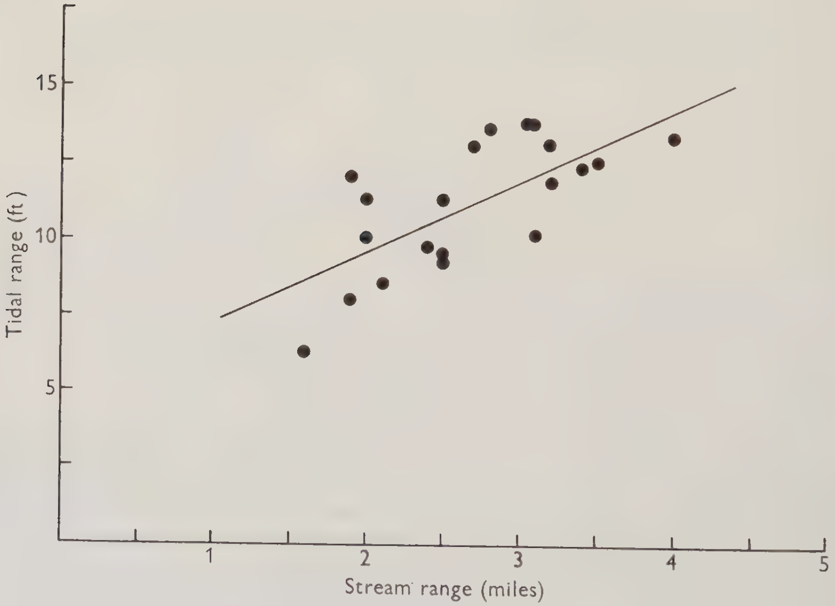


Fig. 5. Stream range compared with tidal range at mid-water.

however, where the wind current is less strong, true stream movement becomes more apparent. Off Northumberland still water is rarely encountered and at the period referred to as slack water the stream, having attained its maximum reach northwards or southwards along the coast, is setting either onshore or offshore. From Table 6 it is seen that while the near-surface water usually turns offshore at each slack water this tendency is modified at mid-water and near the bottom, and as the depth increases a pattern of onshore sets at the end of the ebb and offshore sets at the end of the flood becomes manifest, i.e. the stream ellipse has an anticlockwise sense. This is in agreement with Doodson & Warburg's (1941, p. 179) analysis of the behaviour of a progressive wave having a shelving coast on the right of its direction of travel. Such a pattern suggests that drifting and suspended material would tend to ground at the end of the ebb, which in the region under consideration is about $2\frac{1}{2}$ h after low water at the Tyne entrance, low water and slack water being there separated by this interval.

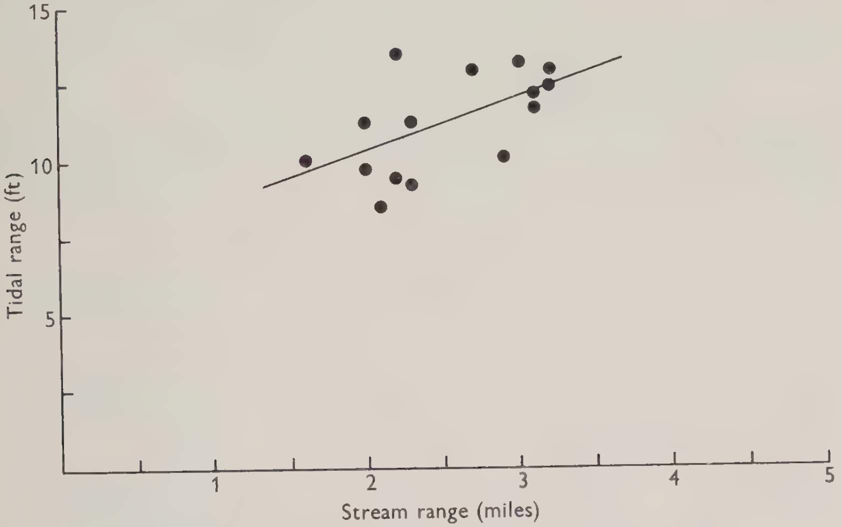


Fig. 6. Stream range compared with tidal range near the bottom.

TABLE 6. 120 OBSERVATIONS OF THE TURN OF THE TIDE OFF NORTHUMBERLAND

	Flood end		Ebb end	
	Offshore	Onshore	Offshore	Onshore
Near surface	20	5	18	7
Mid water	12	8	7	13
Near bottom	10	5	3	12

Both wind currents and tidal streams may effect a permanent displacement of position of planktonic organisms. This is obviously so for wind currents but less so for tidal streams, whose mean vector is zero. In the latter case it is brought about by the combination of differing stream ranges at different levels and the vertical movement of plankton (which, off Northumberland, takes place readily in 10 fathoms of water and less). Even so, certain conditions are necessary to effect such displacement. As an example, let it be assumed that a planktonic organism spends its day at two levels, e.g. near the surface during darkness and near the bottom during daylight. Then no displacement will occur if (*a*) the organism spends the same amount of time at each level or if (*b*) slack water falls half way through its sojourn at either level. The greatest displacement for each factor respectively occurs (*a*) if the organism spends 18 h at one level and 6 h at the other, and (*b*) if slack water falls at ± 3 h from the midpoint of sojourn at either level.

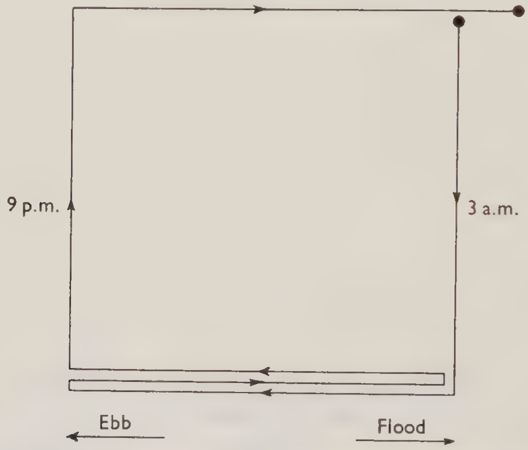


Fig. 7. The tidal stream forces acting on a planktonic organism which migrates vertically at 3 a.m. and 9 p.m. slack waters.

To illustrate this (Fig. 7), with the end of flood occurring at 3 a.m., if a planktonic organism retires to near the bottom at this time and returns to near the surface at 9 p.m. then over a day it will spend two ebb streams and one flood stream near the bottom and one flood stream near the surface. Since stream range is, on average, 0·7 miles greater near the surface than near the bottom it will, in a day and by stream influence alone, be moved this average distance southwards along the Northumberland coast.

In addition it may be noted that tidal streams, whose mean vectors at any one level are zero, may yet affect the spatial separation of pairs of planktonic organisms, provided such organisms undertake vertical migration at different times of day; tidal streams, like wind currents, can thus contribute to patterns of plankton distribution.

With the aid of the foregoing information and armed with records of wind, tidal range and hours of daylight it is possible to plot for a small number of days the movement of plankton along the Northumberland coast, together with some estimate of the error in our final position. Using local weather forecasts we may also attempt short-term prognosis. Over the short intervals envisaged the wind speed/current speed and stream range/tidal range relationships should be employed, not the mean vectors.

TABLE 7. FREQUENCY DISTRIBUTION OF REPRESENTATIVE ANIMALS TAKEN BY VERTICAL HENSEN NET

	Times of hauls and numbers of animals						
	08.00	09.30	11.00	12.30	14.00	15.30	17.00
29 Jan.							
Nyctiphanes couchii	0	0	8	4	0	8	12
Sagitta elegans	0	2	2	6	2	2	2
Calanus finmarchicus	27	10	58	96	99	45	40
12 Feb.							
Nyctiphanes couchii	14	123	259	117	69	197	
Sagitta elegans	8	16	29	30	19	17	
Calanus finmarchicus	198	155	287	277	251	522	
20 Feb.							
Nyctiphanes couchii	3	4	30	76	21	271	103
Sagitta elegans	1	24	35	74	33	38	20
Calanus finmarchicus	23	33	145	269	66	414	254
4 Mar.							
Nyctiphanes couchii	2	0	0	27	5	54	77
Sagitta elegans	1	8	3	9	13	9	8
Calanus finmarchicus	5	2	1	13	33	72	13
11 Mar.							
Nyctiphanes couchii	1	0	0	2	1	5	
Sagitta elegans	0	2	11	13	8	1	
Calanus finmarchicus	2	1	1	3	8	12	

Plankton in the Northumberland seas is unevenly distributed as it is elsewhere. Anraku (1956) has shown that uneven distribution occurs within very small limits of sampling. During the present work small-scale patchiness was again shown during attempts to secure replicate plankton samples from close beside the mid-water drogue. Samples were taken at $1\frac{1}{2}$ h intervals on 5 days, with a Hensen net which was hauled vertically from bottom to surface. By hauling the net close to the mid-water drogue much the same water was fished on each occasion, especially in the deeper regions where the bulk of the plankton was known to be lying. The frequency distribution of a few representative planktonic animals is shown in Table 7. Clearly, even allowing for small changes in depth, the uneven distribution of plankton extends to smaller limits than these.

What follows is presented as a promising but so far poorly pursued line of enquiry into plankton patchiness. It is concerned with the turbulent nature of all sea current flow (Sverdrup, Johnson & Fleming, 1956, p. 90). Turbulence has the effect of altering the spatial relationships of water particles in a random manner; it is that diffusing component of flow (Ludlam & Scorer, 1954) which leads to the dispersal or aggregation of particles in a turbulent medium.

In August 1958 small-scale turbulence in water movement off Northumberland was encountered during an investigation of the accuracy of individual current measurements using drogues. Pairs of drogues used in the studies of gross currents were employed, both drogues being set to ride 6 fathoms below the surface. They were launched together one mile east of Blyth Light in 10 fathoms of water and their tracks were plotted over a period of 2 h. The bearing and distance of one drogue from the other was ascertained by bringing the ship in transit with the buoys and taking a compass bearing and vertical sextant angle of the buoy poles which were 7·5 ft long. Distances were then extracted from table 15 of the Admiralty Manual of Hydrographic Surveying (1952). Runs with pairs of drogues were made on 10 occasions (Table 8); the maximum separation of the two drogues occurred on 15 August and was 840 ft. In view of the identical form of the buoys and drogues their separation can best be explained by invoking the principle of turbulent flow known to be associated with all sea currents.

TABLE 8. SEPARATION OF A PAIR OF DROGUES OVER TWO HOURS

(Separation in feet)

	Time interval (minutes)								
Date	0	20	40	60	80	100	120	Current speed (knots)	Wind speed (knots)
5 Aug.	25	95	80	135	125	140	190	0·5	9
6 Aug.	15	40	60	75	50	65	75	0·2	13
7 Aug.	15	25	40	50	95	130	145	0·2	12
8 Aug.	15	60	45	100	90	130	140	0·2	5
11 Aug.	25	50	60	45	75	85	105	0·3	2
12 Aug.	40	45	70	75	95	85	140	0·3	1
13 Aug.	20	15	0	0	25	60	55	0·2	5
14 Aug.	35	10	40	70	95	145	170	0·4	11
15 Aug.	25	165	155	460	690	660	840	0·7	6
18 Aug.	15	30	5	25	65	110	130	1·0	6

The number of observations is too small for detailed statistical treatment and the results give little more than a qualitative impression of the turbulent forces present. Stommell (1949), in reviewing studies of turbulence, notes that by the so-called '4/3 law' dispersing forces increase as the separation between particles grows. Off Northumberland the separation during the second hour averaged 1·17 times that of the first hour, and the mean separation after 2 h was 200 ft. This suggests that separation after half a lunar day averages at least $\frac{1}{4}$ mile.

From the results of these studies the proposition is advanced that, regardless of other factors, the combination of varying wind currents and tidal streams, turbulent flow, and the vertical migration of much of the plankton ensures that plankton distribution off Northumberland is of a dynamic and irregular nature; and that even when the intrinsic behaviour of the planktonic organisms is left out of account there remains a tendency directed towards patchiness.

My acknowledgements are due to the Coast Guards of Tynemouth for providing wind records. I remain greatly indebted to Skipper R. Harrison of the R.V. 'Alexander Meek' for his extensive co-operation in this work.

SUMMARY

Using free drogues observations have been made of the wind currents and tidal streams at three depths off the Northumberland coast. Records of current speed and direction for the near-surface, mid-water and near-bottom levels are given, together with tidal stream ranges. A relationship is established between wind current and wind, and between stream range and tidal range. Using pairs of drogues the characteristics of oceanic turbulence over a short period were investigated. The relationship of all these factors to plankton patchiness is considered.

REFERENCES

Admiralty Manual of Hydrographic Surveying, 1952. London: H.M.S.O.

ANRAKU, M., 1956. Some experiments on the variability of horizontal plankton hauls and on the horizontal distribution of plankton in a limited area. *Bull. Fac. Fish. Hokkaido Univ.*, Vol. 7, No. 1.

DOODSON, A. T. & WARBURG, H. D., 1941. *Admiralty Manual of Tides*. London: H.M.S.O.

EVANS, F., 1957. Sea currents off the Northumberland coast. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 493–9.

LUDLAM, F. H. & SCORER, R. S., 1954. *Further Outlook*. London: Allan Wingate.

PROUDMAN, J., 1953. *Dynamical Oceanography*. London: Methuen.

STOMMELL, H., 1949. Horizontal diffusion due to oceanic turbulence. *J. mar. Res.*, Vol. 8, pp. 199–225.

SVERDRUP, H. U., JOHNSON, M. W. & FLEMING, R. H., 1946. *The Oceans, Their Physics, Chemistry, and General Biology*. New York: Prentice-Hall.

J. mar. biol. Ass. U.K. (1959) 38, 543–546
Printed in Great Britain

A NEW SPECIES OF COPEPOD FROM THE EDDYSTONE SHELL GRAVEL

By S. Krishnaswamy

Department of Zoology, University of Southampton

(Text-figs. 1–2)

Recent investigations of the bottom fauna off Plymouth by Spooner (1959*a*, *b*) have revealed the existence of a number of unusual and interesting crustaceans. The copepods collected during this investigation were very kindly placed at the disposal of the writer for study. A careful examination of the collection revealed the presence of a second and new species of *Paramisophria* Scott. This genus was established in 1897 by Scott on the basis of females collected in Scotland. Sars (1903) described the male for the first time. *Paramisophria cluthae*, the only species known previously, has been recorded from Plymouth.

Paramisophria spooneri nov.sp.

The Female (Fig. 1 A–L)

Body cyclopoid in shape and slightly compressed laterally. The anterior end of the cephalosome is rounded while the posterior margin is slightly produced and bears a small tooth about its middle (Fig. 1 B). The urosome is short and is about a third of the total length. Caudal rami (Fig. 1 D) are wider than long and each ramus bears six setae. Its inner margin is hirsute.

The antennule is short and only 20 segments could be made out clearly (Fig. 1 C). It carries a number of aesthetes. The basal segment is nearly 4½ times longer than the succeeding joint. The antenna is biramous, both the rami being two-jointed (Fig. 1 F). The endopod is short and the terminal joint which is short carries one apical and two lateral setae. The exopod is long and slender, its terminal joint being slightly shorter than the basal joint, carrying two lateral and four apical setae. Thus the antenna differs from that of *Paramisophria cluthae* Scott, where the endopod is 6-jointed and also carries a larger number of setae. Other oral appendages are as in *P. cluthae*. The maxillule however differs in that it shows a reduction in the number of setae it carries (Fig. 1 G).

The rami of the first four pairs of the swimming feet are 3-segmented. The first pair (Fig. 1 H) is slightly shorter than the other pairs. Basal$_2$ has an outer seta. The first and the second joints of the exopod carry an outer spine and an inner seta each and the terminal joint one apical and two outer spines and four inner setae. The endopod is nearly as long as the exopod and the outer distal corner of the second and the third joints is produced into a spinous projection. The first joint carries an inner seta, the second joint two inner setae and the terminal joint a short plumose outer spine and four inner setae. The seta formulae of the second, third and fourth legs are shown in Table 1.

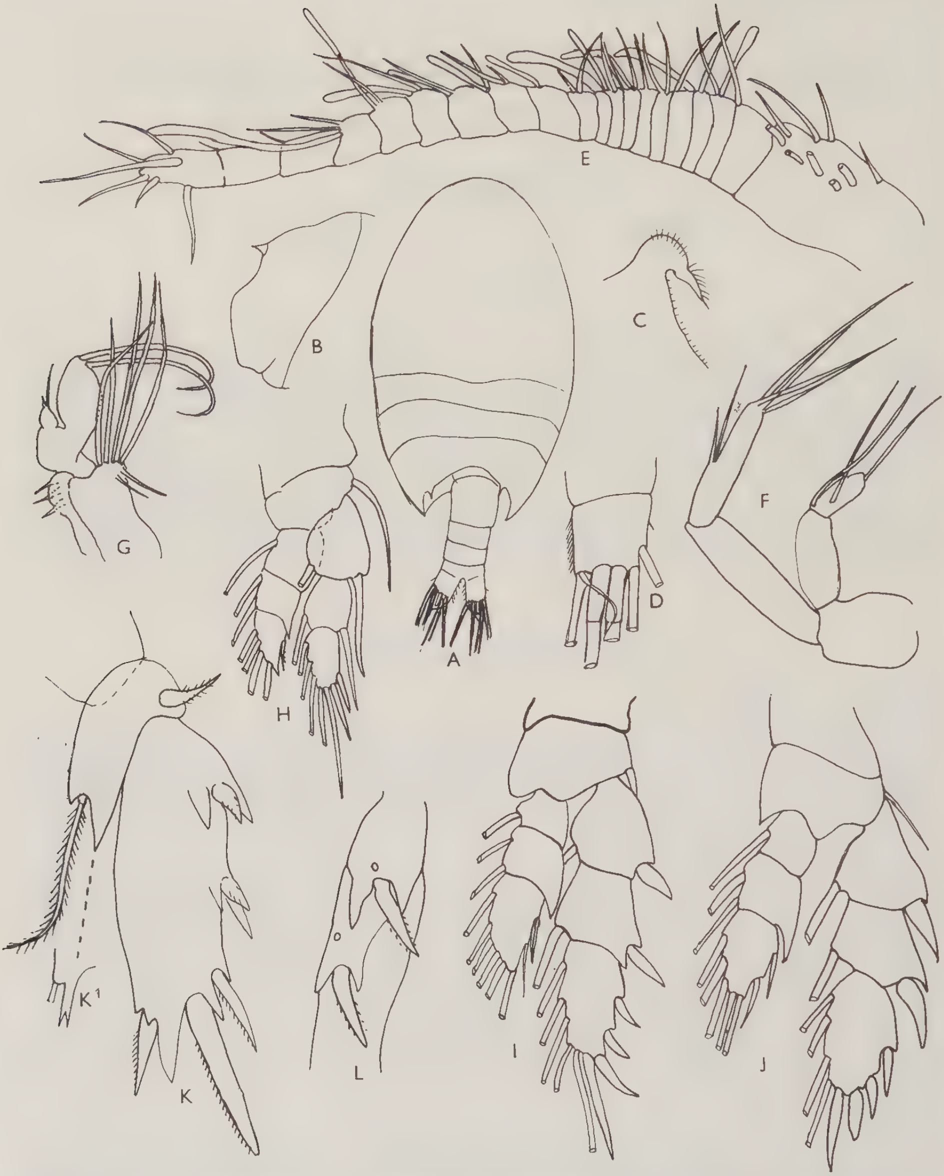


Fig. 1. *Paramisophria spooneri* n. sp. Female. A, dorsal view; B, posterior margin of metasome, lateral view; C, labrum, lateral view; D, caudal ramus; E, antennule; F, antenna; G, maxillule; H, I, J, swimming feet 1, 2 and 3; K, fifth leg; K¹, end of the inner projection with the bifid end; L, lateral view of the fifth leg showing the insertion of spines.

Fifth leg (Fig. 1K) consists of a small basal joint and a long and slender distal joint. The basal joint has an inner as well as an outer seta and is produced on the inner side into a process with a bifid end. The distal joint, which is three times longer than wide, is produced into a spinous process terminally. It carries an outer apical and three outer lateral spines. The outer lateral spines are borne on processes which are produced on either side, as may be clearly seen in a lateral view (Fig. 1L). In *P. cluthae* on the other hand, the inner margin of the basal joint is not produced into a bifid process. Length: 0·62 mm.

TABLE 1

	Exopod		Endopod	
	Outer spine	Inner setae	Outer spine	Inner setae
Second leg	1, 1, 4	1, 1, 5	0, 0, 2	1, 2, 6
Third leg	1, 1, 4	1, 1, 5	0, 0, 2	1, 2, 5
Fourth leg	1, 1, 4	1, 1, 4	0, 0, 2	1, 2, 5

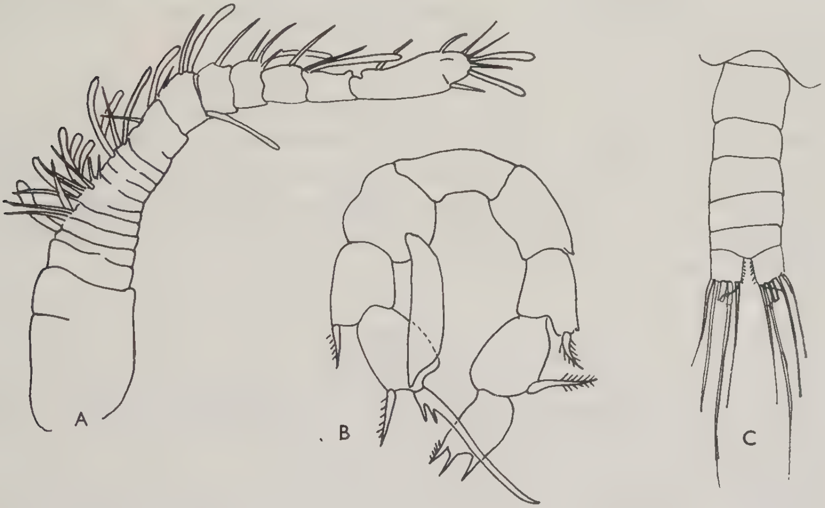


Fig. 2. *Paramisophria spooneri* n.sp. Male. A, antennule; B, fifth leg; C, urosome.

The Male

Resembles the female in general shape of the body. The urosome is however 5-jointed (Fig. 2C). The antennule is 19-jointed and is geniculate on the left side, having the last segment feebly and imperfectly hinged to the penultimate segment (Fig. 2A). The antenna and oral appendages as well as the first four pairs of swimming feet are as in the female. The fifth pair is modified and is 5-jointed (Fig. 2B). On the left the third and the fourth joints carry an outer plumose spine each while the terminal joint which is spatulate is produced into three processes which are spinous, thus giving the appearance of a three-pronged process to the joint. On the right side, the second joint carries a blunt process which is long and reaches up to the end of the fourth joint. The third and the fourth joints carry an outer plumose spine each. The terminal joint is a long slender process with two teeth at its base on the outer side. Length: 0·62 mm.

Remarks

The present species resembles *Paramisophria cluthae* Scott very closely. It differs from it, however, in the structure of the antenna and fifth legs. The presence of a long unarmed blunt process on the second joint on the right side of the male fifth leg is a feature unique for this species. In view of these differences the present species is treated as a new one. I have great pleasure in naming it after Mr G. M. Spooner of the Plymouth Laboratory.

The presence of an inner lobe on the right side of the male fifth leg necessitates the alteration of the generic diagnosis given by Sars (1903, p. 127) to read as follows: 'those in male 5-articulate, *with* or without any lobe inside the 2nd joint'.

Sars (1903) included this genus under the family Arietellidae, and Brodsky (1950) has recently created a new sub-family Arietellinae to include *Arietellus*, *Paraugaptilus*, *Paramisophria* and *Scottula*.

I wish to express my sincere thanks to Mr G. M. Spooner, The Laboratory, Plymouth, for having given me the opportunity to examine this interesting collection and to Prof J. E. G. Raymont, Department of Zoology, Southampton University, for several valuable suggestions. To the Director and Staff of the Marine Biological Association, Plymouth, I am very grateful for the facilities which they have provided.

REFERENCES

Brodsky, K. A., 1950. Calanoida of the far eastern and polar seas of the U.S.S.R. *Fauna of the U.S.S.R.*, Vol. 35, pp. 1–442. (Pub. Inst. Sci. Moscow.)

Sars, G. O., 1903. *An account of the Crustacea of Norway. IV. Copepoda Calanoida.* Bergen Museum, 171 pp.

Scott, T., 1897. The marine fishes and invertebrates of Loch Fyne. *15th Ann. Rep. Fish. Bd Scot.*, Vol. 3, pp. 107–74.

Spooner, G. M., 1959*a*. The occurrence of *Microcharon* in the Plymouth offshore bottom fauna, with description of a new species. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 57–63.

—— 1959*b*. New members of the British marine bottom fauna. *Nature, Lond.*, Vol. 183, pp. 1695–6.

J. mar. biol. Ass. U.K. (1959) 38, 547–556
Printed in Great Britain

THE POLYCHAETE *MAGELONA FILIFORMIS* SP. NOV. AND NOTES ON OTHER SPECIES OF *MAGELONA*

By Douglas P. Wilson, D.Sc.
The Plymouth Laboratory

(Text-figs. 1 and 2)

As already mentioned (Wilson, 1958), a species of *Magelona* from clean sand near low water at Mill Bay, Salcombe, has not yet been described. This worm was first noticed in 1939; it is recorded in the 1957 edition of the *Plymouth Marine Fauna* as '*Magelona* sp.' and it is there mentioned that artificial fertilizations were made and larvae reared in April–September 1939, and that these larvae differed from those of the other two *Magelona* species from the Plymouth district (*papillicornis* F. Müller and *alleni* Wilson). The worm is still common in the same locality at Salcombe, in the same ground and often in the same spade-full as *papillicornis*, but it is not as abundant as the latter and probably not as abundant as it was when first seen in 1939. A very good low tide receding below datum is needed to collect it; it is easily overlooked on account of its fragility and fine thread-like appearance, mature females coloured pink by their contained eggs being more readily seen while digging than translucent immature worms or white males. Worms are difficult to collect whole, the tail end usually being left behind in the sand.

Until recently this worm had only been seen at Salcombe. Now, through the kindness of Mr A. D. McIntyre, specimens of a dwarf variety from inshore waters off the east coast of Scotland have also been studied and are discussed below.

The following description of the species is based on specimens collected at Salcombe in 1939 and recently. Some specimens were simply preserved in formalin, others narcotized in 7% magnesium chloride in tap water and fixed in Bouin's fluid used hot and then preserved in alcohol. Most of the drawings in Fig. 1 are from such fixed and preserved specimens mounted unstained in euparal.

Special thanks are due to Dr Olga Hartman, Allan Hancock Foundation, Los Angeles, for her continued interest in my worm since she first saw specimens in 1939, and for her helpful correspondence then and recently.

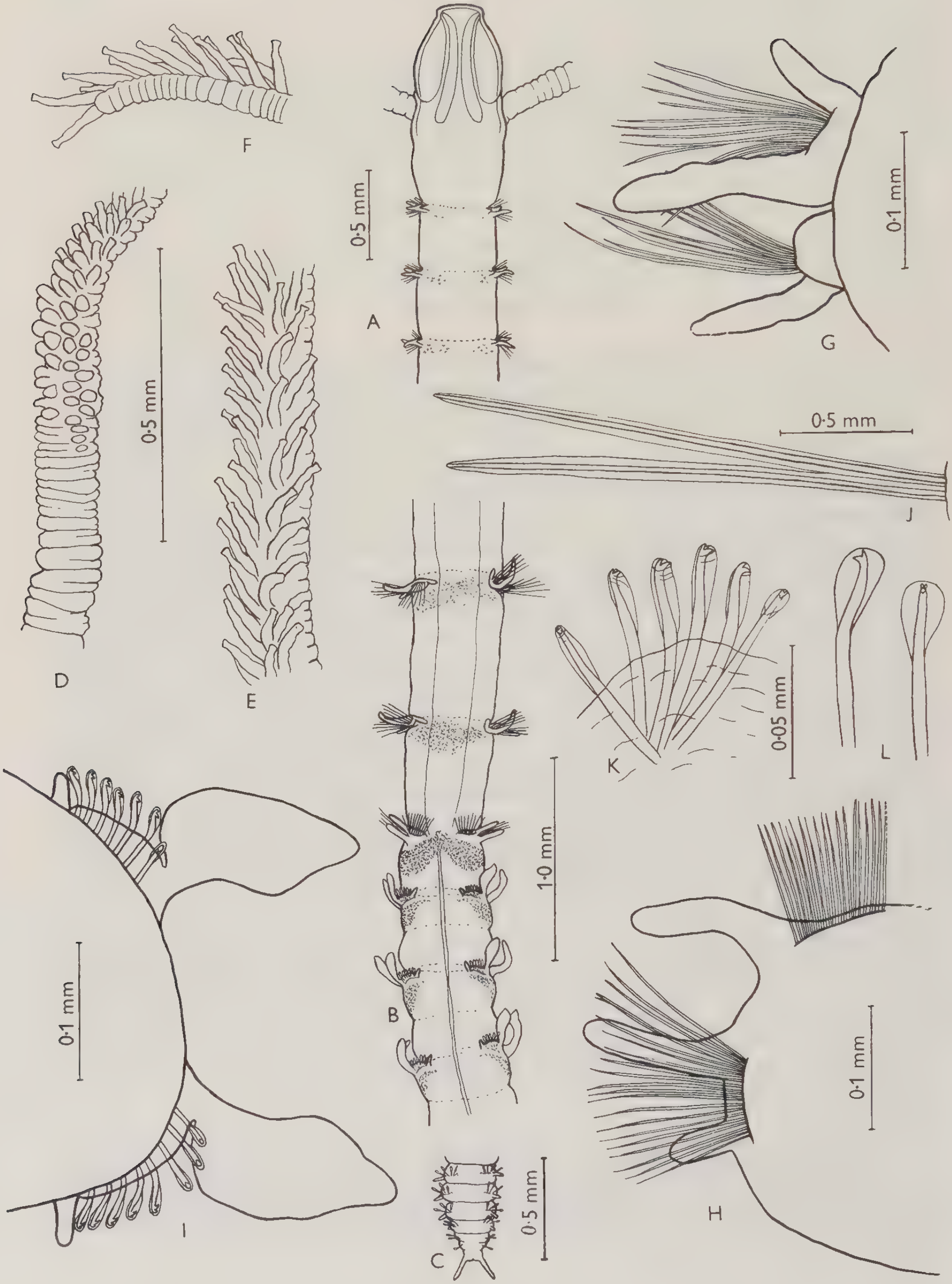

A
0·5 mm
B
1·0 mm
C
0·5 mm
D
0·5 mm
E
F
G
0·1 mm
H
0·1 mm
I
0·1 mm
J
0·5 mm
K
0·05 mm
L

Fig 1. For legend see bottom of facing page.

Magelona filiformis sp.nov.

Adult specimens in the relaxed state reach lengths of more than 80 mm, possibly 100 mm, but are only about 0·3–0·5 mm wide. The width is fairly uniform for most of the body length except that the extreme posterior end tapers gradually to the anus, and there is a slight constriction at the ninth setiger. Total number of setigers in a complete specimen was 142; some individuals probably have more. Prostomium (Fig. 1 A) eyeless, spatulate, longer than wide with two low dorsal longitudinal ridges; anterior border relatively wide, slightly convex and with corners or slight horns on each side. The everted proboscis is globular, longitudinally ridged. On either side of the mouth, below the postero-lateral corners of the prostomium, there arises a long slender tentacle roughly twice as long as the anterior region of the body (in narcotized and fixed specimens). A short proximal portion is transversely wrinkled and without papillae; for most of its length the tentacle carries on one face long capitate papillae arranged in two main rows (Fig. 1 D–F), the other face being as wrinkled as the base.

The first nine pairs of parapodia carry only double-winged bristles (Fig. 1 J); these wings are most readily seen in formalin preserved material examined in water. The first eight pairs of parapodia are all of similar structure (Fig. 1 G), the notopodium with a short dorsal cirrus and a long ventral finger-like process above and below the fifteen (approximately) bristles, the neuropodium with a ventral finger-like cirrus of moderate length and approx. ten bristles. Segmental limits are difficult to observe, the anterior region being largely free from definite annulations. Slight grooving sometimes occurs immediately anterior to the parapodia but sections show that the septa are in advance of these grooves, usually no annulations marking their positions. The parapodia are situated towards the front end of each segment. Parapodia are spaced at progressively increasing distances apart from the first pair to the eighth, the hinder segments being markedly longer than broad. The limits of the ninth setiger are ill-defined externally and need to be worked out in sections. The ninth parapodia (Fig. 1 H) are of special construction; the notopodium has an almost comb-like row of 25–30 fairly straight double-winged bristles and a ventral finger-like process of moderate length; the neuropodium has two finger-like processes, the dorsal moderately long the ventral short, and 25–30 curved double-winged bristles arising from a setal-sac anterior to the processes.

The parapodia of the long posterior region from the tenth setiger backwards are all similar with rather widely separated noto- and neuropodia (Fig. 1, I), each with a foliacious lamella on a short stalk and a row of hooks on a torus dorsal or ventral to it, and a short dorsal or ventral cirrus. The lamellae are approximately equal in size and are slightly larger on anterior than on posterior segments. The hooded hooks each have one large tooth surmounted by two smaller teeth (Fig. 1 K, L). The hoods appear larger and more widely spread in formalin preserved material examined in water (Fig. 1 L) than in fixed specimens mounted in euparal (Fig. 1 K). At the anterior end

---

Legend to fig. 1

Fig. 1. *Magelona filiformis* sp.nov. A, dorsal view of prostomium and first three setigers; B, dorsal view of body in the region of setigers 7 to 12; C, dorsal view of anal extremity; D, E and F, proximal, middle and extreme distal portions of a tentacle showing arrangement of capitate papillae; G, 6th parapodium; H, anterior view of 9th parapodium; I, 13th parapodium; J, emergent portions of two adjacent bristles, from a formalin preserved specimen examined in water; K, some hooded hooks from a specimen mounted in euparal, the full lengths of the shafts not being drawn; L, lateral and front views of hooded hooks from a formalin preserved specimen examined in water.

of the posterior region the intersegmental grooves are ill-defined; they appear to lie mid-way between the parapodia (Fig. 1B). Farther back segmental limits are more clearly visible and in passing back along the body of the worm there is a gradual transformation in the position of the parapodia which come to lie at the posterior end of each segment, which except when contracted or swollen with genital products are markedly longer than broad. The last few segments have incompletely developed parapodia. There are two anal cirri (Fig. 1C).

Living specimens are in general colourless with a transparent body wall through which the gut, pale pink in the anterior region, transparent in the mid region and brownish posteriorly, is seen. In mature specimens pink ova or creamy-white sperm showing through the body wall colour the region of the body in which they lie, that is most of the posterior region except for some of the anterior segments. In a mature specimen swollen genital segments give a moniliform appearance to the worm. Careful examination reveals a pattern composed of patches of epithelia cells with brownish yellow intracellular granules. The dorsal aspect of this pattern is indicated in Fig. 1A and B. The granules occur dorsal to and a little behind the parapodia, reaching their greatest density on a few segments just in front of and behind the transition region between the eighth and tenth setigers, there being a particularly large and dense patch of granular cells just behind each ninth parapodium. On the ventral surface there are more of these cells with granules, at the bases of the parapodia from about the fourth pair and in groups mid-ventrally anterior to the parapodia, and there is again concentration in the region of the ninth parapodia. Farther back they form a thin interrupted band on each side of the mid-ventral line and there are prominent longitudinally elongate patches on the sides of the segments, forming on each side of the worm an almost continuous band of brownish pigment interrupted only at the parapodia.

The following characters in combination are sufficient to distinguish *filiformis* from all other known species: (1) the presence of small prostomial horns; (2) the structurally modified ninth parapodium in which the dorsal cirrus is absent and the bristles are similar to those in front; (3) the finger-like processes of all anterior region parapodia, the first eight pairs with dorsal and longer ventral cirri and the notopodium with an even longer process below the bristles; (4) the widely separated and approximately equal sized foliacious lamellae of the tenth and succeeding setigers.

The typical form is known only from sand (fairly clean to rather muddy) near E.L.W.S.T., Mill Bay, Salcombe, south Devon, in the same ground with *M. papillicornis* F. Müller. It lives in fragile tubes, which may be no more than the walls of burrows lined with a secretion to which sand grains adhere. A minute form (see below) is found in muddy ground off the north-east coast of Scotland.

The above description and drawings are based on several specimens from Salcombe. A specimen has been chosen as the holotype and deposited in the British Museum (Natural History) and given the number 1959.4.2.1. It is a complete worm of 142 setigers in two portions, the last few segments having broken off during preservation. Both portions are mounted in euparal. Other specimens have also been deposited and given the paratype numbers 1959.4.2.2/10.

Magelona filiformis sp.nov. **minuta** var.nov.

McIntyre (1958) has recorded as *M. rosea* Moore, a small magelonid from muddy sand off the north-east coast of Scotland, it being particularly abundant in the Aberdeen coastal area (at one station 26 were found in $\frac{1}{5}$m^2). It was also present in his bottom samples from St Andrews Bay and to a lesser extent from Smith Bank. At my request Mr A. D. McIntyre kindly supplied me with some of his specimens (preserved in formalin). The immediate impression was of their small size and transparent tissues, in these respects recalling to mind large magelonid larvae from the plankton, but they had, of course, been obtained from grab hauls in muddy sand. A typical specimen of 60 segments incomplete posteriorly was 15 mm long and varied in width from 0·20 to 0·30 mm. For comparison 60 segments of a Salcombe worm measured 38 mm long and varied in width from 0·30 to 0·50 mm. Close examination has shown these North Sea worms to agree in structural detail with the much larger *filiformis* specimens from Salcombe, in fact except for size they cannot be separated from them. Mr McIntyre has himself compared them with Salcombe specimens and with tracings from my drawings and he is in agreement with this. He informs me that he has never had any larger specimens than these very small ones, although his collections have been made throughout the year, and he has never had them from the shore or from water of a depth of less than 10 m. These small worms begin to appear with *papillicornis* (which is found in shallower water) at depths a little greater than 10 m; they were common at one station at 18 m and were found down to 59 m. It would appear therefore that these worms are genuinely a small variety and not merely young ones. Unfortunately I have not been able to satisfy myself that genital products are present in any of the specimens I have seen (collected January, April and July), but as they were incomplete posteriorly it is just possible that genital segments had been lost or the products were shed during preservation.

On these small transparent worms the pigmented patches of intracellular granules show up strikingly; by reflected light against a dark background the pigment is yellow rather than brownish yellow, but it follows exactly the pattern already described for the Salcombe worms.

Specimens of this dwarf variety have also been deposited in the British Museum (Natural History).

COMPARISON WITH OTHER KNOWN SPECIES

It has become the practice to divide the genus *Magelona* into two convenient groups, those with prostomial horns and those without. The latter includes *papillicornis* F. Müller, 1858; *obockensis* Gravier, 1906; *rosea* Moore, 1907; *pitelkai* Hartman, 1944*a*; *californica* Hartman, 1944*b*; an unnamed species near *californica* mentioned by Hartman (1951); *alleni* Wilson, 1958. The horned species include *cincta* Ehlers, 1908 (see Wilson, 1958); *pacifica* Monro, 1933; *japonica* Okuda, 1937, and *japonica* var. *koreana* Okuda, 1937; *cornuta* Wesenberg-Lund, 1949; *cerae* Hartman & Reish, 1950. By virtue of its small horns *filiformis* would thus be included in this latter group; parapodial characters clearly separate it from *cincta*, *pacifica*, *japonica*, *japonica* var. *koreana*, and *cerae*. Parapodial characters separate it also from *cornuta*, some notes on which follow.

There remains the doubtful species *longicornis* Johnson, 1901, described by Johnson from two imperfect specimens collected at West Seattle in 1899. It is difficult on the basis of Johnson's incomplete description and poor figures (when he wrote only one other species was known, namely *papillicornis*, and he was easily able to separate his worms from that) to point to clear-cut distinctions between his species and *filiformis*. He mentions that the proboscis lacks corrugations (grooved in *filiformis*) and that the hooks are bidentate (tridentate in *filiformis*). Hartman (1944*b*, pp. 318–19) has discussed *longicornis*; she points out that 'it is not certain whether the prostomium has frontal horns' and discusses an ambiguity concerning the setae. Her conclusion is that *longicornis* be 'regarded as a species *incertae sedis*'. In a private letter (from which she very kindly allows me to quote) she describes the species as unrecognizable, mentioning that there are at least five recognizable species in the north eastern Pacific (excluding Okuda's two species from Japan) and that 'which one of these (if any) might be *M. longicornis* of Johnson from Washington, would be sheer guess, since Johnson left no types and the published account is useless. There may be specimens, so labelled, in the U.S. Nat. Mus. but they can hardly be regarded as type specimens.'

In the British Museum (Natural History) there is a tube of specimens labelled *Magelona longicornis* 1924.5.5.58/62 and I am indebted to the Museum for the loan of this. The inside label indicates that the worms were collected on Pleasant Beach, Seattle, and were determined by F. A. Potts (who probably collected them). This tube contained one nereid worm and five *Magelona*; three of the latter are *papillicornis* or a closely allied species, while the remaining two agree with Johnson's imperfect description and may possibly be the species he saw. An examination of these two worms shows the following features: a prostomium horned a little more prominently than in *filiformis*: proboscis lightly grooved longitudinally; foliacious, not finger-like, lobes below the notopodial bristles; ninth parapodia with foliacious lobes, dorsal cirri but no ventral cirri; lamellae of tenth and succeeding setigers foliacious, dorsal and ventral approximately equal in size and apparently not stalked but springing from broad bases; hooks definitely bidentate, the main tooth being surmounted by a single smaller tooth. In almost all these points these specimens differ from *filiformis*. The two specimens are both incomplete posteriorly; one is of about 30 setigers and measures approximately 23 mm long and has widths of about 1·0 and 1·5 mm in the anterior and posterior regions respectively.

Fauvel (1936, p. 63) has identified as *Magelona rosea* Moore a worm from the Atlantic coast of Morocco, but his figures and description of it do not agree with Moore (1907, pp. 201–4, pl. XVI). Fauvel, for instance, draws a markedly horned prostomium, whereas Moore shows a rounded frontal margin. Fauvel's worm cannot from his figures and description be identified with any known species. *M. rosea* has been recorded by Southern (1914, p. 105) from Killary Harbour, Ireland, largely it would seem because the setae of the ninth setiger tapered to a point. Moore himself confirmed the identification from specimens sent by Southern. Eliason (1920, p. 52) records three fragments of the same species from the Öresund. It is desirable that these records be checked in the light of modern knowledge of the genus.

COMPARISON WITH *MAGELONA CORNUTA* WESENBERG-LUND

M. filiformis bears some resemblance to *M. cornuta* Wesenberg-Lund (1949, p. 328 and fig. 36) from the Gulf of Iran. As in that species each posterior parapodium bears two large foliacious lamellae of equal size and the anterior parapodia as drawn in Wesenberg-Lund's figure appear to be similar to those of *filiformis*. Both species have frontal horns, but those of *cornuta* are much more pronounced. Moreover, *cornuta* is decidedly larger than *filiformis* and is differently coloured, and the hook as shown in Wesenberg-Lund's figure and description ('a blunt bidentate tip') would appear to be of an aberrant type for the genus. However, normal *Magelona* hooks have a similar appearance to that shown in her drawing when they are seen in partial front view. Normally they do not arise from papillate processes as indicated by Wesenberg-Lund's artist, but are arranged in transverse rows dorsally and ventrally. To check these details and to enable a better comparison to be made between what appeared to be two closely similar species I have, through the kindness of Dr Wesenberg-Lund, examined her type specimen of *cornuta*. This has enabled me to add to and amend the original description.

Fig. 2A is a drawing of the eighth parapodia of *cornuta* seen *in situ* in dorso-lateral view, the view presented by the specimen as it lies in a dish. These parapodia seem to be similar to all those anterior to them, more doubtfully so to the ninth parapodia. One of the latter had been removed for the purpose of the original description and the other is difficult to examine in detail without spoiling the specimen. In order to avoid further damage to the specimen I have done no more than examine the parapodia *in situ*, or in microscopical preparations of them loaned to me by Dr Wesenberg-Lund. Examination *in situ* is difficult, but by critically positioning a spot-light most details can be made out. In the eighth and anterior parapodia the notopodial bristles arise between a minute dorsal cirrus and a foliacious lamella of very transparent tissue. On the eighth setiger this lamella is clearly visible on the right side of the worm but on the left it is seen nearly edge-on (see Fig. 2A). The neuropodial bristles spring between two finger-like processes the lower one being the ventral cirrus; in Fig. 2A these are clearly indicated on the left side.

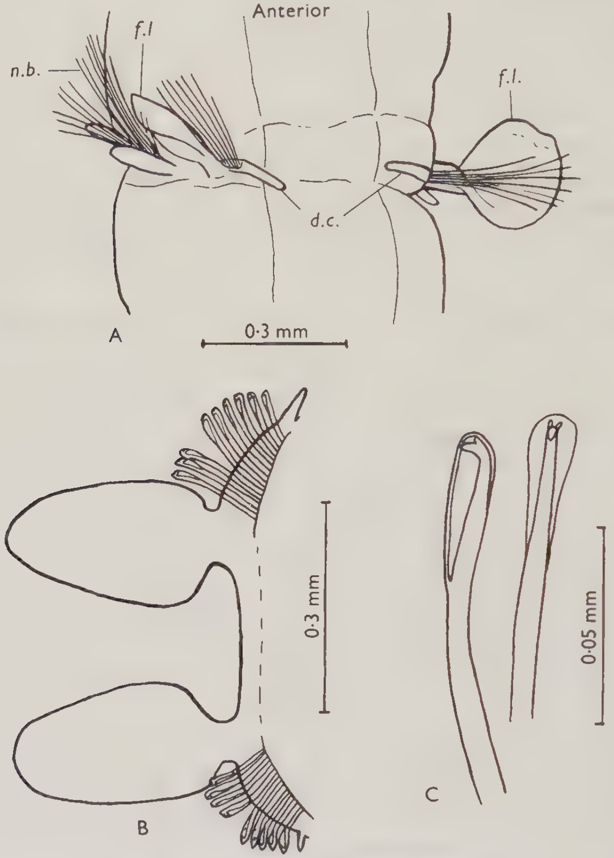


Fig. 2. *Magelona cornuta* Wesenberg-Lund. Some details from the type specimen. A, semi-dorsal view of the eighth pair of parapodia, with the worm heeled over to the right and showing more of the left parapodium than of the right. *d.c.*, dorsal cirri; *f.l.*, foliacious lobe below notopodial bristles; *n.b.*, neuropodial bristles with finger-like process above and ventral cirrus below. B, a parapodium of about the fifteenth setiger. C, hooded hooks in lateral and front views.

In two features therefore these anterior parapodia differ from those of *filiformis* which has a finger-like process below the notopodial bristles and not a foliacious lamella, and which lacks the upper of the two neuropodial processes of *cornuta*. These parapodial differences clearly separate the two species.

The posterior parapodia of the two species are very much alike (compare

Figs. 1, 1 and 2B), as already mentioned. The hooded hooks of *cornuta* are arranged in short transverse rows (about ten hooks to a row) dorsally and ventrally and do not arise from papillate processes (see above). The hooks (Fig. 2C) are tri-dentate; in front view two minute teeth above the main tooth are clearly visible. In partial front view they do look similar to Wesenberg-Lund's figure. A minute dorsal and ventral cirrus at the extremity of each row of hooks can be seen with careful illumination. The large lamellae are extremely transparent.

SUMMARY

A *Magelona* first found at Salcombe in 1939 is described as a new species and given the specific name *filiformis*. It is distinguished from all other species of the genus by its parapodial characters and from some species by its small prostomial horns. So far it has been obtained in its typical form only at Salcombe.

A markedly dwarf variety from inshore waters off the east coast of Scotland is given the variety name *minuta*. Formerly recorded by Mr A. D. McIntyre, who found it, as *M. rosea* Moore it is structurally identical with *filiformis* from Salcombe but differs from it by being very much smaller when adult.

M. filiformis is compared with other species of *Magelona*, in particular *longicornis* Johnson which is shown to have been imperfectly established, and with *cornuta* Wesenberg-Lund, the original account of which is amended and expanded following a re-examination of the type specimen.

REFERENCES

EHLERS, E., 1908. Die bodensässigen Anneliden aus den Sammlungen der deutschen Tiefsee-Expedition. *Wiss. Ergebn. 'Valdivia'*, Bd. 16, Lf. 1, pp. 1–167.

ELIASON, A., 1920. Biologisch-Faunistische Untersuchungen aus dem Öresund V. Polychaeta. *Acta. Univ. lund.*, Bd. 16, No. 6, 103 pp.

FAUVEL, P., 1936. Contribution à la faune des Annélides Polychètes du Maroc. *Mém. Soc. sci. nat. Maroc*, No. 43, pp. 1–143.

GRAVIER, C., 1906. Contribution à l'étude des Annélides Polychètes de la Mer Rouge. *Nouv. Archiv. Mus. Hist. nat., Paris*, Sér. 4, T. 8, pp. 123–236.

HARTMAN, O., 1944*a*. Polychaetous annelids from California including the description of two new genera and nine new species. *Allan Hancock Pacif. Exped.*, Ser. 1, Vol. 10, pp. 239–307.

—— 1944*b*. Polychaetous annelids. Part VI. Paraonidae, Magelonidae, Longosomidae, Ctenodrilidae, and Sabellariidae. *Allan Hancock Pacif. Exped.*, Ser. 1, Vol. 10, pp. 311–89.

—— 1951. The littoral marine annelids of the Gulf of Mexico. *Pub. Inst. mar. sci. Univ. Tex.*, Vol. 2, pp. 7–124.

HARTMAN, O. & REISH, D. J., 1950. The marine annelids of Oregon. *Ore. St. Monogr. Zool.*, No. 6, 64 pp.

JOHNSON, H. P., 1901. The Polychaeta of the Puget Sound Region. *Proc. Boston Soc. nat. Hist.*, Vol. 29, pp. 381–437.

Marine Biological Association, 1957. *Plymouth Marine Fauna*, 3rd ed.

McIntyre, A. D., 1958. The ecology of Scottish inshore fishing grounds. 1. The bottom fauna of east coast grounds. *Mar. Res. Scot.*, 1958, No. 1, 24 pp.

Monro, C. C. A., 1933. The Polychaeta sedentaria collected by Dr C. Crossland at Colón, in the Panama Region, and the Galapagos Islands during the expedition of the S.Y. *St George*. *Proc. zool. Soc. London*, 1933, Pt. 4, pp. 1039–92.

Moore, J. P. 1907. Descriptions of new species of spioniform annelids. *Proc. Acad. nat. Sci. Philad.*, Vol. 59, pp. 195–207.

Müller, F., 1858. Einiges über die Annelidenfauna der Insel Santa Catharina an der brasilianischen Küste. *Arch. Naturgesch.*, 24 jahr., Bd. 1, pp. 211–20.

Okuda, S., 1937. Spioniform polychaetes from Japan. *J. Fac. Sci. Hokkaido Univ.*, Ser. 6 (Zool.), Vol. 5, pp. 217–54.

Southern, R., 1914. Clare Island Survey. Archiannelida and Polychaeta. *Proc. R. Irish Acad.*, Vol. 31 (2), Pt. 47, 160 pp.

Wesenberg-Lund, E., 1949. Polychaetes of the Iranian Gulf. *Dan. sci. Invest. Iran*, Pt. 4, pp. 247–400.

Wilson, D. P., 1958. The polychaete *Magelona alleni* n.sp. and a re-assessment of *Magelona cincta* Ehlers. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 617–26.

J. mar. biol. Ass. U.K. (1959) 38, 557–568
Printed in Great Britain

THE BRITISH SPECIES OF *LUTRARIA* (LAMELLIBRANCHIA), WITH A DESCRIPTION OF *L. ANGUSTIOR* PHILIPPI

By N. A. Holme
The Plymouth Laboratory

(Plates I and II, and Text-figs. 1–4)

The Lutrariidae are large lamellibranchs which burrow deeply in bottom-deposits on the shore and in shallow water. The foot is relatively weak, and they live permanently buried 30 cm or so beneath the surface, maintaining connexion with the overlying water through their long siphons, which are united in a common sheath for practically their whole length. The commonest British species, *Lutraria lutraria* (L.) is found at low-water mark on shores of muddy sand, and offshore its siphons are sometimes taken by the dredge or grab. Another species, *L. magna* (da Costa) is more local, and appears to inhabit muddy deposits near the mouths of estuaries. It is a southern form, being occasionally found on the south and west coasts of the British Isles, and, like *L. lutraria*, its range extends southward to the Mediterranean.

Examination of shells from offshore shell-gravel deposits near Plymouth has revealed the presence of a third form, which appears to correspond with the *L. elliptica* var. *angustior* of Philippi (1844), a variety subsequently noted by several other authors under different names. Although a distinct form, it has never been assigned specific rank, perhaps because many of the specimens found would have been dead shells washed up on the beach, in which the hinge-teeth, an important character for identification, are usually worn or broken. This form was taken by Ford (1923, 1925) at Plymouth, but was identified as *L. magna*, and I have also collected specimens from off other parts of the south coast of England (see Appendix for list of localities).

LUTRARIA ANGUSTIOR PHILIPPI

?*Mactra lutraria* variety. Turton, 1819, p. 85.
Lutraria elliptica (form). Brown, 1827, plate xii, fig. 3.
Lutraria elliptica (form). Brown, 1844, plate xliii, fig. 3.
Lutraria elliptica var. *angustior* Philippi, 1844.
Lutraria elliptica var. *intermedia* Sowerby, 1859, plate iv, fig. 1, *nec.* fig. 2.
Lutraria elliptica var. *alterutra* Jeffreys, 1863.
Lutraria elliptica var. *angustior* Philippi. Hidalgo, 1870, plate 6, fig. 2.
Lutraria elliptica var. *attenuata* Monterosato, 1878.
Lutraria lutraria var. *angustior* Philippi. Bucquoy *et al.* 1896.

A form of *L. lutraria* with a shell narrower than the type has been noted by a number of authors, but where the description is brief or unaccompanied by an illustration, some doubt must remain as to the identity of the specimen. Descriptions in the literature do not indicate that the hinge-teeth or pallial scar are different from those of typical *L. lutraria*, so that identity with the form here described is based solely on the shape and proportions of the valves. The first illustrations of *L. angustior* are in Brown (1827, 1844), while those of Sowerby (1859), Hidalgo (1870), and especially Bucquoy *et al.* (1896) are also of the same species. The description, unaccompanied by a figure, by Jeffreys (1863) of var. *alterutra* corresponds with that of *L. angustior*, but although he mentions Philippi's record of *L. lutraria* from Sicily he evidently does not regard the variety as synonymous with Philippi's var. *angustior*. Philippi's description is brief, giving dimensions of the shell, and the opinion that it is intermediate between *L. lutraria* and *L. magna*.

Philippi's original specimens are apparently no longer in existence, but his opinion that it is intermediate between the two other species strongly suggests that it is the species described here. Bucquoy *et al.* (1896) give the most detailed description, accompanied by photographs, of *L. lutraria* var. *angustior*, based on specimens from the French Mediterranean coast, but unfortunately do not illustrate or describe characters on the inside of the shell. These authors considered that the variety they describe corresponds not only with Philippi's description but with that of specimens from British waters described by Brown, Sowerby and Jeffreys. This lends additional support to the use of Philippi's name *angustior* for the British specimens described here.

Shell

The shell is equivalve, and of an oval-elongate shape, the specimen shown in Pl. I, figs 2 and 3, measuring 11 cm long × 5·9 cm high. The ventral and posterior dorsal margins are moderately straight and parallel with one another. The posterior end is fairly evenly rounded, but the anterior end is usually more sharply curved towards the dorsal side (Fig. 1 B). The valves gape slightly in front, and more so posteriorly, but the arrangement of the hinge in all members of the genus is such that the valves can rock longitudinally about the hinge cartilage, so that the relative gape at either end can be varied. The shell is moderately thick, and is marked on the outside with concentric striae corresponding to the lines of growth. The periostracum on the smallest specimens is colourless and transparent, on larger specimens it is brown and slightly glossy, but on all the adults examined it was totally abraded, exposing the white prismatic layer. The beaks are not very acute, and are scarcely directed forward. They are situated about two-fifths of the shell's length from its anterior end. The ligament, as in the other species, is small and mainly internal. There is no lunule and the escutcheon is virtually absent, as in the other species.

The inside of the shell is white and slightly glossy, with well-marked muscle scars and pallial line. There is a deep U-shaped pallial sinus which extends slightly forward of the mid-point of the length of the shell. The scar marking the lower edge of the sinus runs almost parallel and close to the ventral pallial scar for the greater part of its length, the two usually merging for a part of the length of the sinus. The range of variation of this character is between that shown in Fig. 1B and that in Pl. I. The distal end is usually marked by an oval enlargement of the scar. The hinge plate is fairly flat, and bears a triangular pit to accommodate the large cartilage. The posterior dorsal edge of this pit is almost straight, and makes an angle of about 15° with the adjacent dorsal margin of the shell. Each valve bears two erect cardinal teeth. In the right valve these are both laminar and diverge from one another at rather less than a right angle. The first tooth curves downwards so that distally it makes an angle of 60–80° to the second tooth. The first tooth consists of two imbricating teeth, one lying beyond the other, and both point in the same direction. In a worn shell they may appear as a single tooth. Behind the cartilage is a low lateral tooth set in an oval-elongate depression. There is no anterior lateral tooth. The inner dorsal margins of the shell are not grooved, as in *L. lutraria*, and the dorsal surfaces tend to curve inwards so that the outer, striated, surface is visible in a lateral view of the interior (Fig. 2B).

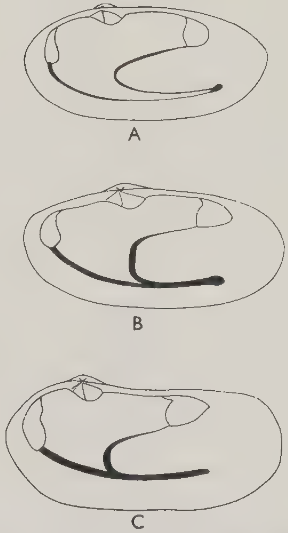


Fig. 1. Interior of the right valve of *Lutraria* shells, showing muscle and pallial scars. A, *L. lutraria*; B, *L. angustior*; C, *L. magna*.

The two cardinal teeth in the left valve engage between the cardinals of the right valve; the first is slightly convex on its outer side, the second straight. On their inner sides the teeth are thickened, and they are fused together for about a third of their lengths. There is a thin tongue-shaped tooth at the side of the cartilage pit, behind the second cardinal tooth. It is present in all three species (see Pl. II), but is broken off in the majority of dead shells collected. Behind the cartilage pit is a low lateral tooth, as in the right valve, and there is a curved anterior lateral tooth running almost parallel with and outside the first cardinal tooth. Both lateral teeth are set in shallow depressions, but the remainder of the inner dorsal margin is convex.

Siphons

This description is based on a single whole adult specimen, and on a number of siphons cut off by the dredge. Since the siphons are all that may be captured on offshore grounds, the differences between those of the three species are of some importance for identification.

The siphons of all Lutrarias are very long, and can be extended to two or three times the length of the shell. The two tubes are united in a common sheath almost throughout their lengths, the sheath being invested with a transparent gelatinous covering which is an extension of the periostracum of the shell. In cross-section the sheath is oval with a slight groove or 'waist' between the two siphons, which are of about the same cross-sectional dimensions.

In *L. angustior* the proximal four-fifths or so of the sheath is a uniform creamy yellow colour, becoming marked with small strawberry-red spots distally. Beyond these there is a circular band of a uniform dark red, followed in many examples by a pale whitish band, the extreme tips of the two siphons being again spotted with strawberry red. The colour pattern varies with individuals, but the dark red band is present in all examples. The two siphons are separate and slightly diverging at their tips (Fig. 3B and Pl. I), and along the dorsal and ventral sides of the common sheath there is a pale line running through the red markings to the tip of each siphon. The siphon openings are fringed with tentacles, but as these are only to be seen when the living animal is fully extended and not disturbed in any way, they are of little use for identification. In this species the dorsal siphon is fringed with numerous small tentacles, while the ventral siphon has eight (or perhaps nine) longer tentacles. There are probably small tentacles between the longer tentacles, as in *L. lutraria*, but these were not distinguished. In *L. lutraria* there are two circles of smaller tentacles outside the opening of the ventral siphon. These were not observed in the living specimen of *L. angustior*, but it cannot be said with certainty that they are absent in this species.

LUTRARIA LUTRARIA (L.)

Shell

The shell grows a little larger than in the previous species, a fairly large specimen measuring 13 cm. long × 7·2 cm. in height. The dorsal margins are more convex and the anterior end more evenly rounded, so that the outline is more nearly elliptical (Fig. 1A). Some specimens, however, have shell proportions similar to those of *L. angustior*. The valves gape to about the same extent as in that species. The shell substance is a little thinner, and the outside rather smoother. The periostracum in all but the smallest shells is an olive-brown colour, glossy, and in addition to the concentric lines of growth, is marked towards the umbo with faint radial lines, which are of use in identifying small specimens (p. 564). The beaks are more acute than in *L. angustior*, and are situated at about the same position relative to the shell's length.

The pallial scar differs from that of *L. angustior*. The scar marking the lower edge of the sinus meets the ventral pallial scar in a narrow V, the two remaining well separated until they meet at the posterior end of the sinus (Fig. 1 A). At this point the scar tends to be expanded as in *L. angustior*. Even in narrow shells having similar proportions to *L. angustior* the two scars remain well separated.

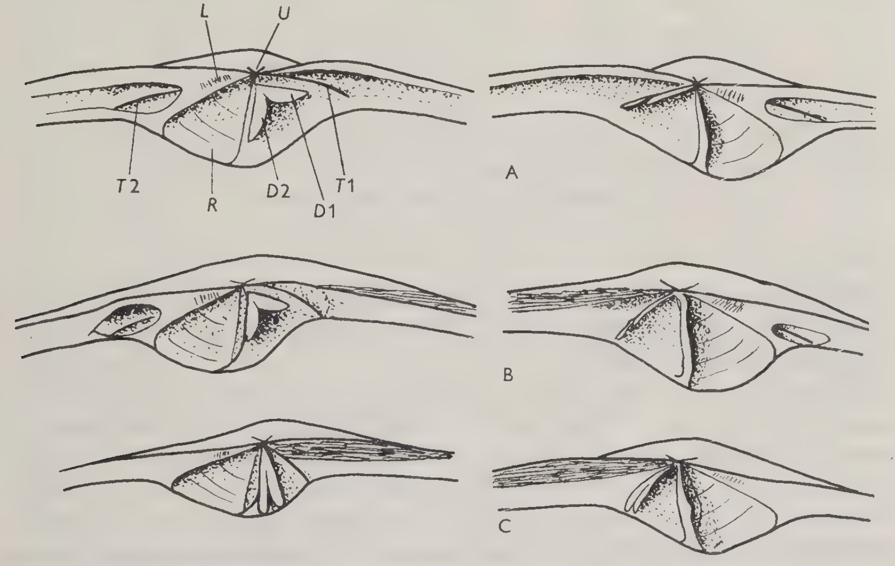


Fig. 2. Hinges of the left and right valves of *Lutraria* shells. The linguiform tooth between the second cardinal tooth and the cartilage in the left valve has been omitted in each case as it is so frequently broken off. *D* 1, *D* 2, 1st and 2nd cardinal tooth; *T* 1, *T* 2, 1st and 2nd lateral teeth; *L*, ligament attachment area; *R*, cartilage pit; *U*, umbo. Note that in no case is a pair of valves from a single specimen drawn, so that the left and right valves drawn do not exactly correspond. *A*, *L. lutraria*; B, *L. angustior*; C, *L. magna*.

The hinge (Fig. 2 A) differs only in detail from the preceding species. The posterior dorsal outline of the cartilage pit is straight or slightly convex, and makes a greater angle (about 30°) with the adjacent dorsal margin of the shell. In the right valve the two cardinal teeth are straight and diverge at 80°–90°. The first tooth is made up of two imbricating teeth in line, as in *L. angustior*. There is a low posterior lateral tooth, but the depression in which it is set is continued posteriorly as a shallow rounded groove inside the dorsal margin of the shell. A similar but rather deeper groove runs forwards from the umbo, passing dorsally to the first cardinal tooth. The dorsal edges of the shell are produced upwards and inwards in this species, so accentuating the depth of these grooves.

In the left valve the two cardinal teeth diverge at a slightly greater angle than in *L. angustior*, and the first tooth is straight on its dorsal side. There is a low anterior and posterior lateral tooth, and the inner dorsal margin of the shell is grooved, as in the right valve.

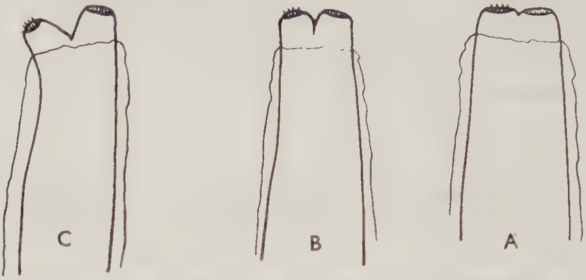


Fig. 3. Siphons of *Lutraria*. In each case the ventral siphon is to the left. A, *L. lutraria*; B, *L. angustior*; C, *L. magna* (after Deshayes).

Siphons

The siphons of this species are described and figured by Deshayes (1844–8, plates xxxv, xxxvii). There is some doubt, however, of the identity of the specimens shown in plate xxxiv.

The siphons of *L. lutraria* are broadly similar to those of *L. angustior*. The proximal two-thirds to three-quarters of the sheath is a uniform yellow or creamy-white, but distally the colour spots are larger and more distinct than in *L. angustior*, and are of a purplish-brown colour (Pl. II). The spots tend to run into one another to form longitudinal markings. Towards the tips of the siphons there is a ring of almost pure white, marked with small purple-brown spots, and the pale lines along the dorsal and ventral sides of the sheath are very distinct. The siphons are scarcely separated, and do not diverge, at their tips (Fig. 3A). The form of the tentacles at the tips of the siphons is described by Deshayes.

LUTRARIA MAGNA (DA COSTA)

Shell

The shell is more elongate than in the other species, a fairly large specimen measuring 12 cm. long × 5·2 cm. high. The umbo lies rather far forward, being about one-quarter of the shell's length from the anterior end (Fig. 1C). The ventral edge is fairly evenly curved, and the dorsal edge behind the umbo is curved upwards to about the same extent, so that the posterior dorsal outline is concave. The anterior end is evenly rounded, but the posterior is obliquely truncate. The posterior gape is much wider than in the other species. The valves are moderately thick, and are covered by a dark brown periostracum. The outer surface is rather irregular, and is marked with concentric growth lines. An indefinite line from the umbo to the ventral side of the truncated posterior divides the surface into a smoother anterior and a more wrinkled and irregular posterior part. The beaks are rather obtuse, and are directed if anything slightly forward.

There is deep U-shaped pallial sinus, the scar marking the lower edge of the sinus merging completely with the ventral pallial scar for practically the whole length of the sinus (Fig. 1C). The distal end of the scar is not enlarged as in the other species.

The posterior dorsal outline of the cartilage pit is straight or slightly concave, and lies at a small angle (*ca.* 10°) to the adjacent dorsal edge of the shell. In the right valve the first cardinal tooth is cloven longitudinally into two subequal halves (Fig. 2C). It is therefore totally different from the corresponding tooth in the other two species. The first cardinal tooth curves downward so that the axis of its more distal part lies at only 30–40° to the second tooth, which is laminar, as in the other species. In both valves the anterior part of the cartilage pit is more deeply excavated than the remainder. Posterior lateral teeth are absent in both valves, and the inner dorsal surfaces are flat or convex. In front of the umbo in both valves the striated outer surface is curved inward to an even greater degree than in *L. angustior*, so that it practically merges with the inner dorsal surface of the shell. In the left valve the two cardinal teeth are almost straight on their outer surfaces, and diverge at 30–40°. Their inner surfaces are fused together for about two-thirds of their length. There is a low anterior lateral tooth, as in the other species.

Siphons

These are described by Deshayes (1844–8, plate xxxvii), and I have not myself seen any specimens. The sheath is white proximally, becoming a shade of purple posteriorly, which gradually increases in intensity towards the tips of the siphons, often terminating in a very deep reddish-purple colour. Coloration is uniform, and there are no colour spots as in the other species, and the pale lines along the dorsal and ventral edges of the sheath are absent. The two siphons are separated at some little distance from their tips, and diverge from one another at almost a right angle (Fig. 3C). The tentacles at the tips of the siphons are a little different from those of *L. lutraria*, and the two outer circles of small tentacles on the ventral siphon are lacking.

IDENTIFICATION OF ADULTS

Identification of *Lutraria magna* presents no problem, as it may at once be recognized by the longitudinally cleft first cardinal tooth in the right valve, the more acute angle between the two cardinal teeth, the absence of posterior lateral teeth in both valves, and by the completely linear scar below the pallial sinus.

It is difficult to find a single character which will invariably separate *L. angustior* from *L. lutraria*. Where the periostracum is intact, the presence of fine radial striae in the older parts of the shell (not to be confused with wrinkling in the later *brown* periostracum) is a sure recognition character for *L. lutraria.* The longitudinal grooving of the inner dorsal surface in *L. lutraria* and the shape of the pallial scars, although showing some degree of variation, are also valuable characters. The degree of curvature of the first cardinal tooth in the right valve, the angle between the teeth in this valve, and the outline of the shell are all rather variable, and should only be used in combination with the more diagnostic characters noted above. Additional characters are the more pointed umbo of *L. lutraria*, and the greater thickness of the shell in *L. angustior.*

IDENTIFICATION OF YOUNG STAGES

I have not examined small specimens of *L. magna*, but this species should be readily identifiable at all sizes from hinge characters, particularly the form of the first cardinal tooth in the right valve.

Mr Ford's collection contains small shells (length range 3–22 mm) of *L. lutraria* and of another form (labelled *L. magna*) from shell-gravel deposits in Plymouth Sound. The latter appears to be *L. angustior.*

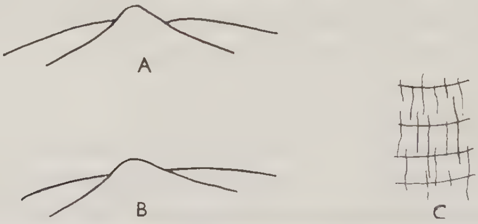


Fig. 4. Identification of young stages. A, lateral view of umbo and dorsal side of left valve of *L. lutraria* (4–5 mm long); B, similar view of *L. angustior*; C, radial markings on periostracum of *L. lutraria.*

At these sizes the shells are white, glossy, and translucent. Comparison of the two species shows that some of the specific differences between the adults are of no use in identifying the young stages. For example, the pallial line is difficult or impossible to distinguish at this stage. While the hinge of small *L. lutraria* is similar to that of the adult, that of *L. angustior* differs in that the two cardinal teeth diverge at almost a right angle, and the inner dorsal surfaces of the shell are grooved as in *L. lutraria.* The form of the cardinal teeth is explained by the fact that the first tooth in the right valve is curved in the adult, and actually diverges from the second at nearly a right angle, so that the angle between the teeth in a young shell would be greater than in the adult. The grooving of the inner dorsal surfaces is discussed in the next section.

The two species may, however, be distinguished by:

(i) The shape of the beak, which is more acute in *L. lutraria* (Fig. 4A, B).

(ii) The presence of irregular *radial* striae in the periostracum of *L. lutraria* (Fig. 4C), these being absent in *L. angustior*.

STATUS OF *L. ANGUSTIOR*

It is evident that *L. magna* is a species quite distinct from the other two, the question at issue being whether *L. angustior* is a variety of *L. lutraria* or a separate species.

Since *L. angustior* inhabits shell gravel while *L. lutraria* inhabits muddy sand, some of the differences between them might be attributed to environmental influences. The looser and possibly more disturbed substratum inhabited by *L. angustior* may require a greater degree of burrowing activity, which might be shown, for example, in a shell which was relatively narrower than in *L. lutraria*. Greater activity might also affect growth of the shell along the inner dorsal surface, so producing a convex rather than a concave surface in larger specimens. However other differences remain, notably the presence of fine radial markings in *L. lutraria*, the angle between the cardinal teeth, the form of the pallial scar, and the colour and degree of separation of the siphons, which cannot readily be attributed to environmental effects.

In spite of a certain variability of some characters there appears to be no intergrading between the two forms, and it therefore seems reasonable to infer that *L. angustior* is a separate species, the name being taken from Philippi's (1844) description of a variety of *Lutraria elliptica* from Sicily.

I am indebted to Mr A. W. Battin for assistance with some of the drawings, and to Mrs E. A. Peace for taking the photographs for Plates I and II. Much of the material was obtained by the Plymouth Laboratory's research vessels, and I am grateful to their captains and crews for their assistance in making these collections.

Note added in proof

Through the kindness of Dr H. A. Rehder of the U.S. National Museum, Washington, I have received photographs of a pair of valves of *L. elliptica* var. *alterutra* from the Jeffreys collection. These leave no doubt as to the identity of Jeffrey's variety with the species *L. augustior* described here. Details of locality are lacking, and this lectotype has been recatalogued under the reference USNM Cat. No. 622510.

Dr R. Kilias of the Zoologisches Museum der Humboldt-Universität, Berlin, has kindly loaned me a pair of valves of an adult specimen labelled '*Lutraria elliptica* var. *augustior* Phil. Sicilien. Benoit. 6223'. These are of particular value as they come from the same country as Philippi's specimens. The shell material is a little thinner and is less abraded than in the British specimens, suggesting that the habitat was more sheltered from wave or current disturbance. The shells agree with the description of *L. angustior* given in this paper in most particulars, and since the periostracum is intact it is possible to see that the fine radial markings on the outer surface are absent, so confirming that the specimens are not *L. lutraria*. The only significant difference is in the dorsal edges, which are slightly produced upwards and inwards so that the inner surfaces are slightly concave. The longitudinal grooves so formed are not as deep as in *L. lutraria* however, and this upward extension of the shell is no doubt the normal condition when living in more tranquil conditions.

SUMMARY

Two species of the genus *Lutraria*, *L. lutraria* (L.) and *L. magna* (da Costa) are well known in British waters. To these is added a third, originally described as *L. elliptica* var. *angustior* by Philippi, but which is considered sufficiently distinct to merit specific rank. A description of the third species, to be called *L. angustior* Philippi, is given, and differences between it and the other two species described. Small specimens taken by Mr Ford in Plymouth Sound and identified as *L. magna* are considered to be *L. angustior*, so that there are at present no records of *L. magna* from the Plymouth area.

REFERENCES

BROWN, T., 1827. *Illustrations of the Conchology of Great Britain and Ireland.* 52 plates. London.

—— 1844. *Illustrations of the Recent Conchology of Great Britain and Ireland, with the Descriptions and Localities of all the Species, Marine, Land and Fresh Water.* 2nd edition. 144 pp. London.

BUCQUOY, E., DAUTZENBERG, P. & DOLLFUS, G., 1896. *Les Mollusques Marins du Roussillon.* Tome II. Fasc. XXIV, pp. 541–620. Paris.

DESHAYES, M. G. P., 1844–48. *Histoire Naturelle des Mollusques. Tome 1. Mollusques Acéphalés.* Exploration scientifique de l'Algerie pendant les années 1840, 1841, 1842. Texte 609 pp, Atlas, 160 pp.

FORD, E., 1923. Animal communities of the level sea-bottom in the waters adjacent to Plymouth. *J. mar. biol. Ass., U.K.*, Vol. 13, pp. 164–224.

—— 1925. On the growth of some lamellibranchs in relation to the food supply of fishes. *J. mar. biol. Ass., U.K.*, Vol. 13, pp. 531–59.

HIDALGO, J. H., 1870. *Moluscos Marinos de España, Portugal y las Baleares.* Tomo III and Tomo IV (Atlas).

HOLME, N. A., 1950. The bottom fauna of Great West Bay. *J. mar. biol. Ass., U.K.*, Vol. 29, pp. 163–83.

JEFFREYS, J. G., 1863. *British Conchology.* Vol. II, 466 pp. London.

MONTEROSATO, MARCHESE DI, 1878. Enumerazione e sinonima delle Conchiglie Mediterraneo. *G. Sci. nat. econ. Palermo*, Vol. 13, pp. 61–115.

PHILIPPI, R. A., 1844. *Enumeratio Molluscorum Siciliae cum Viventum Tum in Tellure Tertiaria Fossilium.* Vol. 2, 303 pp. Halle.

SOWERBY, G. B., 1859. *Illustrated Index of British Shells.* 1st edition. London.

TURTON, W., 1819. *A Conchological Dictionary of the British Isles*, 272 pp. London.

APPENDIX

RECORDS OF *L. ANGUSTIOR*

Philippi's type locality is in Sicily, and Bucquoy *et al.* (1896) record it from the beaches of La Franqui and Leucate. Records from British waters attributed to this species are to be found in Turton (1819), Brown (1827, 1844) and Jeffreys (1863).

Localities from which I have recorded *L. angustior* are as follows:

St Austell Bay, Cornwall

50° 18·7′ N, 04° 44·8′ W. Muddy gravel, 11 m. One small living specimen. 16/11/50.

50° 19·9′ N, 04° 43·8′ W. *Lithothamnion* gravel, 9 m. One small living specimen. 16/11/50.

Plymouth area

Eddystone *Amphioxus* shell gravel. Occasional living specimens; siphons and dead shells often taken. 42–47·5 m.

Mewstone shell gravel. Dead shells and siphons. *ca.* 27 m. March, 1959.

Plymouth Sound. Living specimens at several stations in shell gravel. *See* Ford (1923, as *L. magna*).

Great West Bay

Station 6 (*see* Holme, 1950). Muddy sand and gravel, 19 m. One small living specimen (listed as *L. lutraria*). 29/7/48.

Dawlish Warren

A pair of valves (? living) washed up on beach. 3/2/50.

Weymouth Bay

50° 36·95′ N, 02° 22·9′ W. 18 m. Dead shells. 11/11/58. 50° 37·0′ N, 02° 20·6′ W. 18 m. Dead shells. 11/11/58. 50° 36·4′ N, 02° 19·75′ W. 19 m. Dead shell. 11/11/58. 50° 34·6′ N, 02° 14·45′ W. 27 m. Dead shell. 11/11/58. 50° 34·0′ N, 02° 08·7′ W. 26 m. Dead shell. 11/11/58.

Swanage Bay

50° 37·65′ N, 01° 53·7′ W. *ca.* 18 m. Dead shells. 28/1/59.

Additional records, added in proof

Torbay

Several, living, washed up at Three Beaches, Goodrington after E. gale. 3/4/58.

Falmouth Bay

One small living specimen at each of two stations:

50° 06·65′ N, 05° 03·4′ W. *ca.* 18 m. *Lithothammion* gravel. 17/7/59.
50° 07·2′ N, 05° 04·3′ W. *ca.* 37 m. *Lithothammion* gravel. 17/7/59.

Mount's Bay

One small living specimen at:

50° 06·3′ N, 05° 30·3′ W. *ca.* 22 m. Gravel and stones. 16/7/59.

EXPLANATION OF PLATES

Plate I. *Lutraria angustior*

Fig. 1. Interior of right valve. Dead shell, Swanage Bay. × 1·2.
Fig. 2. View with foot and siphons moderately extended. Eddystone shell-gravel. × 0·65.
Fig. 3. Dorsal view. Eddystone shell-gravel. × 0·75.

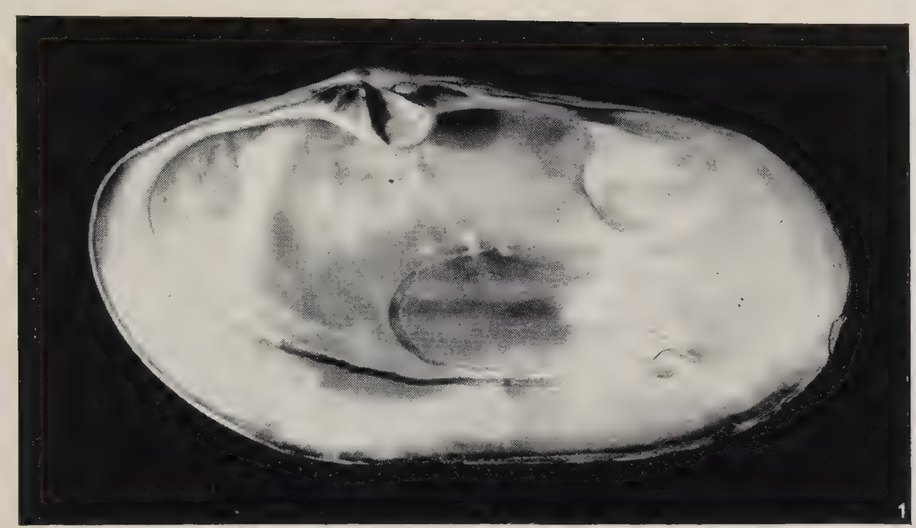
Plate II

Fig. 1. *L. lutraria.* Interior of right valve. From a living specimen. Millport. About natural size.

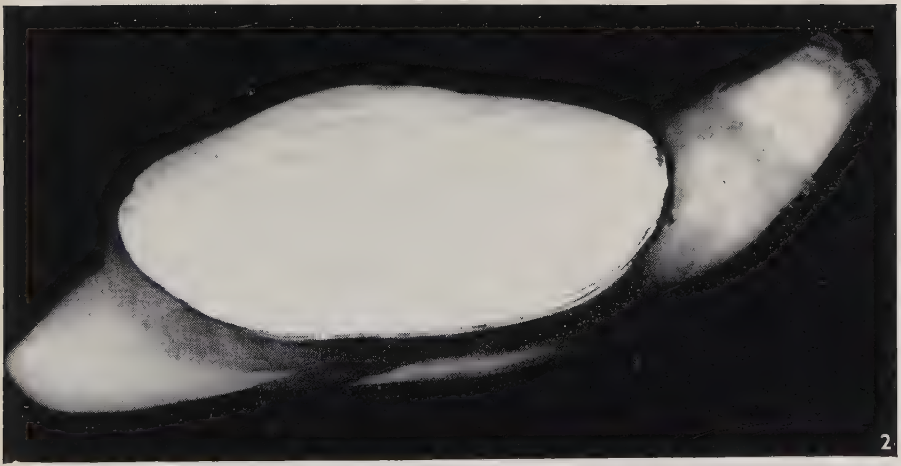
Figs. 2, 3. *L. lutraria.* Hinges of left and right valves. Note the accessory tooth, often broken off, in the left valve behind the 2nd cardinal tooth. From living specimens. Millport. × *ca.* $1\frac{1}{3}$.

Figs. 4, 5. *L. angustior.* Hinges of left and right valves. Dead shells. Swanage Bay. × *ca.* $1\frac{1}{3}$.

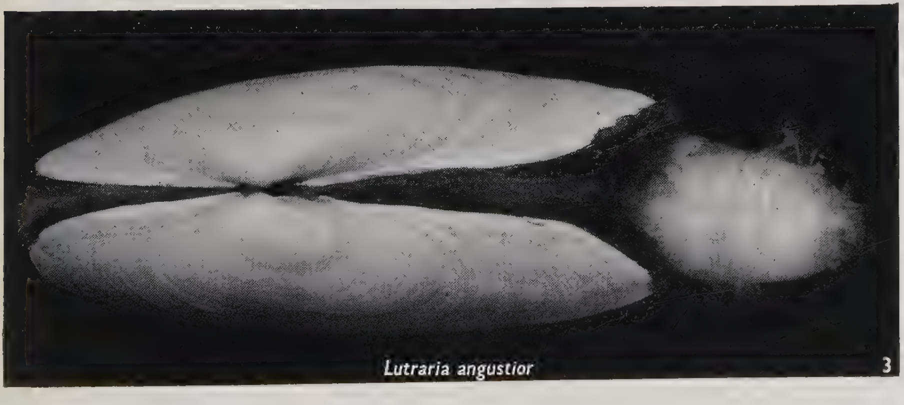
Fig. 6. *L. lutraria.* Posterior part of shell, and partially extended siphons. The dorsal side is uppermost. Cornwall. × 0·87.



1



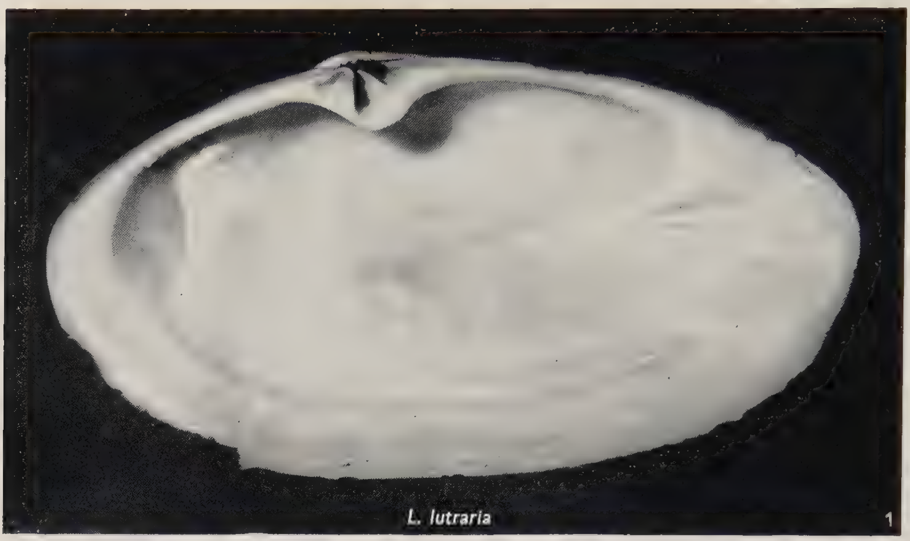
2

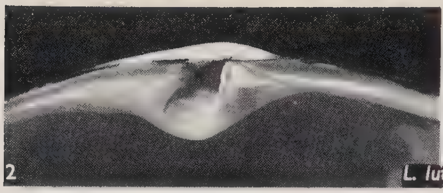


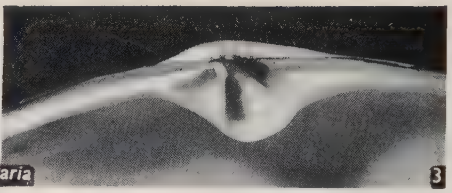
3

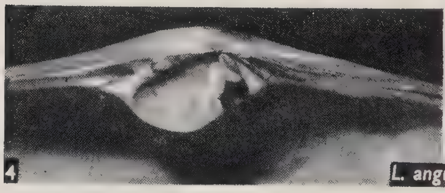
Lutraria angustior

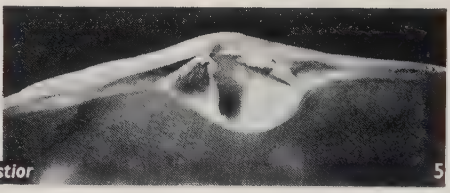
(*Facing p.* 568)


L. lutraria
1


2
L. lutraria


3


4
L. angustior


5

L. lutraria
6

J. mar. biol. Ass. U.K. (1959) 38, 569–580
Printed in Great Britain

THE REACTIONS OF THE LIMPET, *PATELLA VULGATA* L., TO CERTAIN OF THE IONIC CONSTITUENTS OF SEA WATER

By D. C. Arnold

Gatty Marine Laboratory, The University, St Andrews*

(Text-figs. 1–8)

It has been shown (Arnold, 1957) that when exposed to air the common British limpet, *Patella vulgata* L., reacts to splashing with normal or moderately diluted sea water by raising the front edge of the shell, and that the amount of this movement can be correlated with the degree of dilution and with the tide level from which the animals are obtained. A basically similar result was obtained by the use of solutions of sodium chloride. The precise factors influencing the limpets were not identified. Additional data can now be presented on these and other reactions of *P. vulgata* as a contribution towards further definition of these factors.

MATERIALS AND METHODS

The limpets were obtained from a rocky ledge lying just below H.W.O.N.T. at the Pier Rocks, St Andrews, and were collected by chipping away fragments of the rock to which they were attached. In the laboratory they were placed in running sea water until they moved from the shattered rock. Each was then placed in a small polythene cup. Though they could easily climb out, the majority adhered firmly and remained within the cups for several days. Difficulties due to horizontal movement of the animals, encountered in the previous experiments, were thus largely avoided. Specimens which at any stage were found to be damaged were discarded. The shell length of those used ranged from 36 to 47 mm, mean length 41 mm.

As in the earlier work, vertical movements were normally recorded by means of a heart lever attached to a hook inserted beneath the front edge of the shell. An upward movement of the shell was thus recorded as an upward movement on the trace. The presence of a hook caused a local retraction of the mantle fringe, but seemed not to affect qualitatively the behaviour of the animal. A slower drum speed (0·048 mm/sec) and longer lever (magnification 35:1) were used than in the preceding experiments. Stimulation was provided by dropping about 2 ml. of fluid onto the apex of the shell, a small amount passing beneath its edge and coming into contact with the mantle fringe. The amount of liquid used in this manner was found not to be critical; presumably once the space beneath the shell had been filled any excess drained off without effect. Stimuli were customarily given at 5 min intervals, though in a few experiments 10 min intervals were used. After each stimulus the limpets were washed with about 5 ml. distilled water in order to remove any of the stimulating liquid still adhering to the shell. Holes drilled in the bases of the polythene cups allowed these to drain between each test.

---

* Present address: Department of Biology, Wayne State University, Detroit.

The solutions used (sodium chloride, calcium chloride, magnesium chloride, potassium chloride, sodium sulphate, sodium bicarbonate and an artificial sea water composed of these salts) were made up from Analar reagents to the concentrations given by Pantin (1946) as approximately isotonic with sea water of salinity 34·6‰.

All experiments were performed in daylight during the first three days after collection of the limpets. Further details of the methods employed are given in the accounts of the experiments concerned.

PATTERN OF MOVEMENT OF UNSTIMULATED ANIMALS

Despite all care it was impossible fully to prevent vibrations from reaching the experimental animals. A number of records of otherwise unstimulated limpets were therefore made in order to assess how far they might be affected by these unavoidable mechanical stimuli. It was found that under the conditions generally prevailing in the laboratory the limpets, as upon the shore, slowly raised their shells so as to form a slight gap between the edge and the substratum, then remained quiescent except for a series of, usually small, downward movements. The periodicity and amplitude of these contractions varied between different animals (Fig. 1). Occasionally, those animals which had not been long out of water showed considerable activity, and for this reason a period of 8–10 h dryness prior to each experiment was adopted as standard procedure throughout the rest of the investigation. The occurrence of the movements made by the unstimulated limpets could not be related to the general vibrations of the laboratory. Indeed, almost identical traces were given by two animals, one recorded on a day when the laboratory was entirely empty, the other during a visit by a large party of school-children. Only jarring of the apparatus or of the bench upon which it stood produced an unequivocal response, invariably a sharp downward movement. However, care was needed to avoid sudden decreases in illumination, to which the limpets responded in a similar manner. The presence or absence of this shadow reaction formed a useful test for their general well-being during experiments.

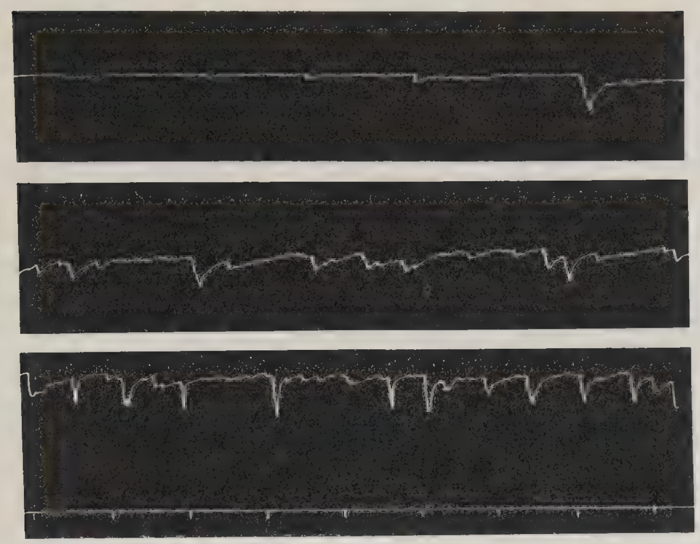


Fig. 1. Part of kymograph records of vertical movements of unstimulated limpets. Time marks at 15 min intervals. Shell lengths 43 mm (upper), 36 mm (middle) and 37 mm (lower).

MODIFICATION OF THE PATTERN THROUGH STIMULATION

Since the limpet is not fully contracted against the substratum when at rest, it may respond to splashing either by an upward movement of the front edge of the shell or by a downward movement. An upward movement was given to sea water, to isotonic solutions of sodium chloride, calcium chloride and magnesium chloride, and to artificial sea water; a downward movement was given to distilled and tap water and to solutions of sodium sulphate, potassium chloride and sodium bicarbonate isotonic with sea water.

Effect of experimental conditions

Since the conditions under which the experiments were performed might be expected to affect the behaviour of the limpets, a series of comparisons were made between animals left undisturbed within the collecting area and those brought into the laboratory, using the responses to sea and fresh water as a guide. It was found that animals upon the shore reacted in a manner qualitatively similar to those studied in the laboratory. Accurate measurements of their movements could not be made, however, and for this purpose a number of specimens attached to small boulders were brought into the laboratory and studied under a binocular dissecting microscope fitted with a micrometer eyepiece. It was thus possible, though tedious, to measure fairly accurately the heights to which the limpets rose upon stimulation, without removing them from their scars or disturbing them in any further manner. The effect of a hook beneath the edge of the shell and of the tension exerted by the heart lever could also be studied in this manner.

It was found that the magnitude of the responses to sea water, measured from the position of maximal contraction of the animal, showed great regularity, while their time relations were also regular. No attempt was made to record these in detail, but most responses were completed in approximately 2 min. Limpets left on their scars gave rather smaller responses than did those re-settled in polythene cups (Table 1). The presence of the hook naturally prevented complete contraction of the animal upon stimulation with fresh water and in these cases the downward movements might be somewhat irregular. In the absence of the hook the limpets always contracted completely when irrigated with fresh water.

TABLE 1. THE EFFECT OF EXPERIMENTAL CONDITIONS UPON THE RESPONSES OF *PATELLA* TO STIMULATION BY SEA WATER

	A	B	C	D
Number of stimuli	20	20	19	10
Mean height attained	1·0	1·5	2·6	1·8 mm
Standard deviation	0·24	0·27	0·22	0·11 mm

A. 38 mm limpet undisturbed on small boulder.
B. Same specimen attached to lever, but still on scar.
C. 40 mm limpet attached to lever and resettled in polythene cup.
D. 36 mm limpet, conditions as in C.
Measurements of A and B made under binocular microscope, those of C and D calculated from kymograph traces.

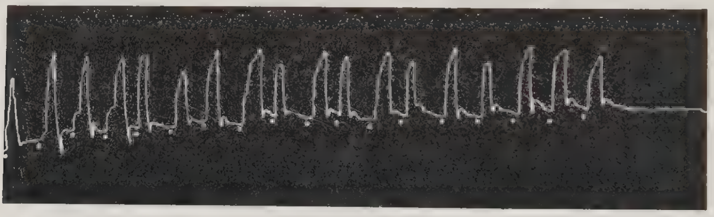


Fig. 2. Kymograph record of response to sea water. Stimuli every 5 min. Shell length 40 mm.

Response to sea water

The response to sea water had certain well-defined characteristics, the majority of which are shown in Fig. 2. The movement commenced abruptly and soon after stimulation and consisted of a smooth, rapid rise to a clear maximum, followed by an equally rapid fall. Occasionally this 'peak' response was preceded by a very small initial contraction of the order of 0·1 mm actual movement. A noticeable feature of the response was that the movements, except for the first one or two in a series, were extremely regular and showed but little variation in their form, general time relations or height attained (by no means the maximum height to which the animals were capable of moving) and the amount of the subsequent downward movement. Under continued stimulation, about half the limpets studied showed a tendency to slight progressive increase in the height of the responses, accompanied by a gradual diminution in the amount of the subsequent downward movements, while the others gave responses of even size. None showed decreased height of movement on repeated stimulation.

Effect of osmotic pressure

As described in the earlier account, the height of response given to sea water diminished with dilution. In order to determine whether this was due to alteration of the ionic concentration or whether it could be ascribed simply to alteration of the osmotic pressure, limpets were tested with normal sea water and with sea water the relative salinity of which had been reduced to 80, 60, 40 and 20% of normal by dilution with either distilled water or 0·9 M dextrose solution. Both series of dilutions, though of different osmotic pressures, elicited responses which showed considerable similarity in size and form at each level, while differences in height between the two series (Fig. 3) were not consistent.

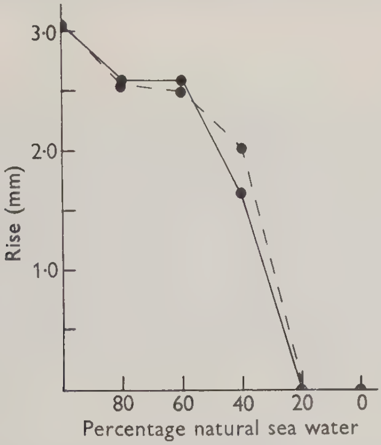


Fig. 3. Response to sea water diluted with distilled water (continuous line) or 0·9 M dextrose solution (broken line). Both distilled water and 0·9 M dextrose solution caused contraction when applied to the extended limpet. Shell length 39 mm.

Influence of pH

Since the reaction of sea water alters slightly upon dilution, while the other solutions used varied considerably in their pH, the influence of this factor was determined by comparison of the reactions to sea water and to isotonic sodium chloride solutions, the pH of which was varied by addition of small amounts of hydrochloric acid or sodium hydroxide. The results of a typical experiment are given in Table 2. In experiments where more alkaline media were used the responses remained regular up to pH 9·5. At pH 10 upward movements were given to the first 2–3 stimuli only, later stimuli eliciting downward movements. Above pH 10·5 the limpets always contracted strongly upon stimulation. If the pH was lowered by use of sulphuric in place of hydrochloric acid the limpets normally moved downward when stimulated and any upward movements given were always smaller than were the responses to sea water.

TABLE 2. EFFECT OF DIFFERENT pH VALUES UPON RESPONSES OF A SINGLE *PATELLA*

	Sodium chloride solutions. Approximate pH					Sea water	
	2·5	4	5	7·5	8		
No. of stimuli	5	5	5	5	5	5	5
Mean height attained	2·1	1·9	1·9	1·9	2·2	2·2	2·1
Standard deviation	0·18	0·13	0·22	0·22	0·22	0·18	0·13

Short series were used with long rests to minimize variability inherent in stimulation with NaCl. The sea-water series were taken at the beginning and end of the experiment. Shell length 40 mm.

Response to sodium and calcium chlorides

The response to sodium chloride solution isotonic with sea water resembled that given to sea water itself in that the mean height of a series of responses was usually of the same magnitude for limpets of similar size. However, many irregularities and distortions appeared in the records and became especially pronounced towards the end of a long sequence of tests (Fig. 4). Most of the upward movements obtained with this form of stimulation were peaks, but both they and the subsequent downward movements showed much greater variation in height than did those given to sea water, while after a time the rapidity of the response was also affected and the movements began to lose their well-defined form, thus giving a transition to a form of response more aptly to be described as a 'hump'. Sodium chloride alone was not an entirely favourable stimulus, since when the isotonic solution was placed upon a portion of the mantle fringe there was a sharp local contraction. After about the tenth stimulation by sodium chloride the shadow reaction was lost, while after about the twentieth stimulation the limpet ceased to react further to either sodium chloride or fresh water and reacted in a quite irregular and unpredictable manner when stimulated with sea water.

The use of isotonic calcium chloride solution as a stimulus resulted in an upward movement, but one of very different form to that elicited by sodium chloride or by sea water. The first few responses were all of the peak type, but thereafter the movements slowed and the maxima became less well-defined, while the subsequent downward movements became slower and smaller until after about the tenth stimulation the limpets failed to react further to either calcium chloride or fresh water (Fig. 5), remaining in an expanded condition with a wide gap between shell and substratum. Responses to calcium chloride were very nearly as regular in the height attained, measured from the level of maximum contraction, as were those given to sea water. The mantle fringe gave no local reaction to isotonic calcium chloride solution.

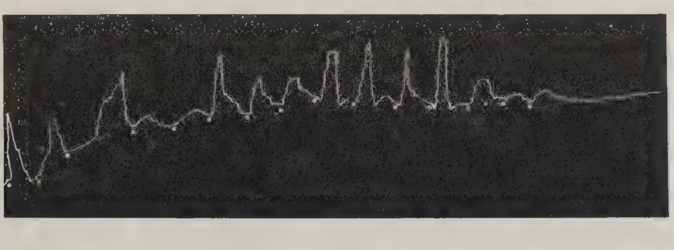


Fig. 4. Kymograph record of response to isotonic NaCl solution. Stimuli every 5 min. Shell length 40 mm.

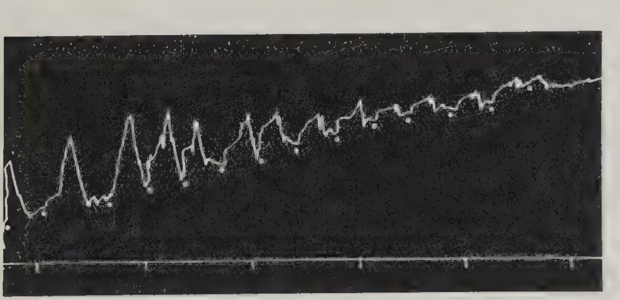


Fig. 5. Kymograph record of response to isotonic $CaCl_2$ solution. Stimuli at 5–10 min intervals. Time mark every 15 min. In this and following records the level of the time mark has been adjusted to the position of maximum contraction of the limpet. Shell length 42 mm.

Since the response to sodium chloride solution resembled that to sea water in its general form, while that to calcium chloride solution did so in its regularity of height attained, it was of interest to examine the effect of mixtures of these two solutions. This proved a matter of some difficulty. The continued use of either one alone was already known to result in characteristic anomalies of behaviour and ultimate cessation of response, while during a long series of tests upon the same animal fatigue and the possible residual influence at each stage of the experiment of the mixture used in the preceding stage had to be considered. The method finally adopted was to group the stimuli in sets of 6 for each test and to alternate each set with a similar group of sea-water stimuli as control. Thus each result could be immediately compared with the behaviour to natural sea water and the stability of this behaviour during the course of the experiment could be assessed. Since such an experiment would involve some scores of stimuli, the limpet was left untouched for 30 min between each set of responses. The combinations of chlorides used were made up from the original solutions isotonic with sea water and contained 1·5, 2·5, 3, 5 and 20% calcium chloride.

An example of a series of traces obtained from a single limpet is given in Fig. 6. The response to sea water showed the same pattern throughout the experiment. The height above base, indicated by the time-trace, remained constant for each upward movement, as did the amount of the subsequent downward movements. Individual peaks were narrow, indicating rapidity of movement, and had clear-cut points (Fig. 6A). Response to an artificial sea water showed a similar pattern, but with the peaks two or three times as wide, indicating a corresponding slowness of movement (Fig. 6B). The pattern of response to isotonic sodium chloride solution resembled the two foregoing patterns in certain respects, but was markedly irregular in the heights attained at each upward movement (Fig. 6C).

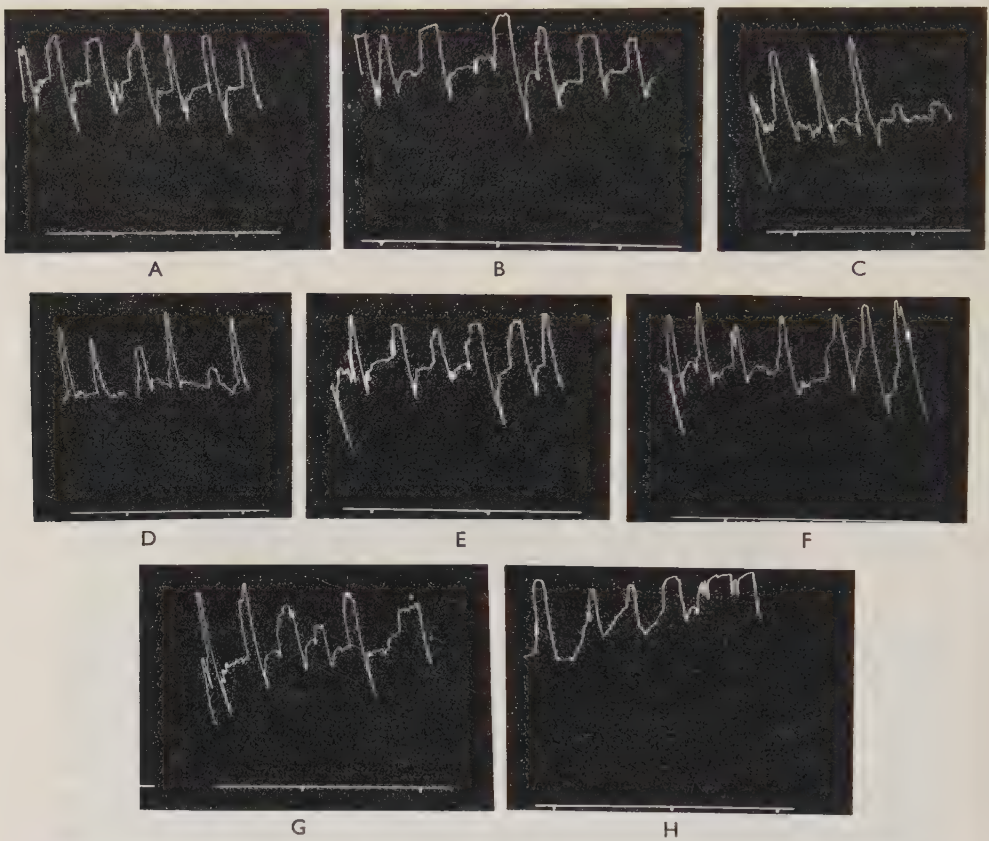


Fig. 6. Kymograph record of short sequences of responses to solutions of varying calcium content. For explanation, see text. Stimuli every 5 min. Time mark every 15 min. Shell length 40 mm.

When isotonic sodium and calcium chloride mixtures were used, that containing 1·5% calcium chloride resulted in a pattern similar to that induced by sodium chloride alone (Fig. 6D). Responses to mixed solutions containing 2·5 and 3% calcium chloride more closely resembled the responses to sea water (Fig. 6E and F). The solutions which contained 5 and 20% calcium chloride induced patterns of movement closer to that elicited by stimulation with isotonic calcium chloride solution alone, namely slowing of all movements and reduction of the downward component with successive stimuli, though the heights attained with the upward movement remained regular (Fig. 6G and H). The trace shown in Fig. 6G is more irregular in the heights attained than was usual for limpets stimulated in this manner; as it was actually the last test conducted in this experiment the pattern may have been modified by fatigue.

By calculation, the mixture containing 2·5% calcium chloride solution, considered to give the best approximation to the pattern of movement characteristic of sea water, contained 0·36 g/l. Ca″, while the artificial sea water should contain 0·40 g/l. Ca″. The limpets might therefore be adapted to a slightly lower concentration of calcium ion than is normally present in sea water. To check this hypothesis the calcium content of the artificial sea water sample used and of inshore water at the time the experiments were conducted (mid-June, 1958) were determined by the method of Kirk & Moberg (1933). The artificial sea water was found to contain 0·41 g/l. Ca″, while two samples of sea water contained 0·37 and 0·38 g/l. Ca″ (inclusive of strontium). These values differ from those for the open sea (Harvey, 1955), but explanation of the discrepancy is beyond the scope of this paper.

Response to magnesium and potassium chloride

Magnesium chloride solution, isotonic with sea water, induced an upward movement similar to that given to sodium and calcium chlorides, but marked by its own specific characters (Fig. 7). The heights attained in response to successive stimuli were quite regular and the movements themselves reasonably smooth and fairly rapid. For a while at least the amount of each downward movement was also stable. However, each upward movement was succeeded by a period in which the limpet remained extended and almost motionless and the onset of contraction was often much delayed, though rapid enough when once it started. As the experiment proceeded these characteristics became ever more pronounced until after about the tenth stimulus a superficial narcosis ensued. In this condition the limpet would respond, albeit slowly, to direct mechanical stimulation of the foot, but it no longer reacted to fresh water, reduction of light intensity or jarring of the bench or apparatus.

In contrast, isotonic potassium chloride solution dropped upon a slightly expanded limpet induced an immediate and complete contraction, followed by a rapid sequence of abrupt, jerky, up-and-down movements, after which the majority of limpets tested in this manner climbed out of the polythene cup and so terminated the experiment.

The effect of absence of magnesium and potassium ions was tested by use of artificial sea waters in which their chlorides were replaced by an osmotically equivalent amount of sodium chloride. These replacements did not have any effect upon the size of the responses as compared with those given to natural and the complete, artificial sea water.

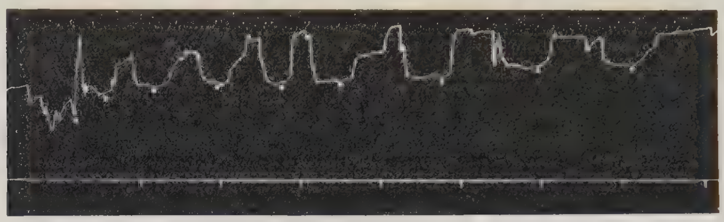


Fig. 7. Kymograph record of response to isotonic $MgCl_2$ solution. Stimuli at approximately 10 min intervals. Time mark every 15 min. Shell length 41 mm.

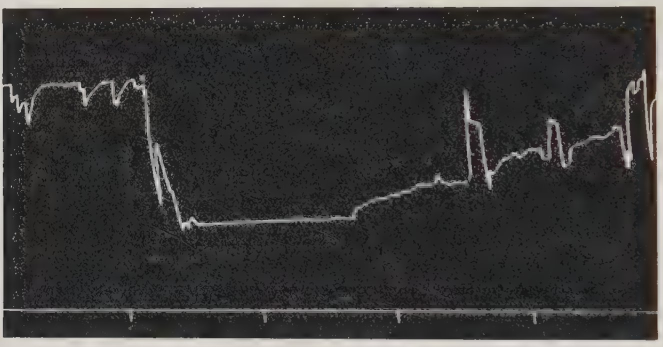


Fig. 8. First part of kymograph record of response to isotonic Na_2SO_4 solution. Stimulus given at *x*. Time mark every 5 min. Shell length 37 mm.

Response to sodium carbonate and sulphate

Isotonic solutions of these two salts induced downward movements. With sodium carbonate the limpet contracted as far as possible upon stimulation, then began a series of fairly rapid up-and-down movements which gradually died away into the normal steady state with its periodic small contractions. With sodium sulphate solution as stimulus the initial contraction was followed by a prolonged period of inactivity (Fig. 8), succeeded by a series of rapid up-and-down movements which might continue for two or more hours before the normal pattern of the quiescent animal was restored.

DISCUSSION

The experiments here described have confirmed the earlier results and show that the responses of *Patella vulgata* to natural and diluted sea water cannot be attributed to recognition of variation in either pH or osmotic pressure of the media. Further, the occurrence of similar reactions when a simple artificial sea water is used as stimulus shows that the responses are not due to the influence of minor ions since, apart from contaminating traces of arsenic and iron in the reagents used, these would be absent. Upward movements have been obtained with simple chloride solutions (except with potassium chloride), but not with sodium sulphate or carbonate, and it follows that the reactions may most easily be attributed to sensitivity to the concentration of chloride ions. Calcium ions apparently exert a stabilizing influence when in optimum concentration and the behaviour of the animal soon indicates whether the amount of calcium present is above or below that of the inshore water to which it is accustomed.

The response of *P. vulgata* to dilution of the external medium differs in its nature from that found in other animals so far investigated. The estuarine lamellibranch *Scrobicularia plana* (da Costa) exhibits behavioural changes when placed in sea water diluted with distilled water, but is unaffected when dilution is secured by means of 0·9 M sucrose solution (Freeman & Rigler, 1957). Loss of activity is shown by the copepod *Tigriopus fulvus* (Fischer) when subjected to salinities of 90‰ and upwards, recovering when the medium is once again diluted (Ranade, 1957), but it is not clear whether the reaction denotes sensitivity to osmotic pressure or to ionic concentration.

The effects of various ions upon *P. vulgata* show interesting similarities to those found for animals belonging to other groups. Thus the branchiopod *Artemia salina* (L.), placed in sodium chloride solution isotonic with sea water survives for several days, but in other solutions the survival time is greatly reduced, the animal becoming moribund in similar solutions of magnesium and calcium chlorides (6–9 h), potassium chloride (30 min) and sodium bicarbonate (5 min) (Croghan, 1958). The importance of calcium ions in resistance to reduced salinity has been demonstrated (Pantin, 1931) for the turbellarian *Procerodes ulvae* (Oersted).

The ecological importance of these reactions in *Patella* can be in little doubt, for they provide a means whereby the feeding period of the limpet may be prolonged beyond the time during which the animal is covered by the tide. In the first place, they enable *P. vulgata* to take advantage of spray cast by breaking waves or carried by an onshore wind; but more than this, the sensitivity to chloride ions and tolerance to a lowered concentration provide a mechanism which would allow the limpet to utilize those periods when the rocks are damp through high humidity or light rain, while ensuring that the animals do not leave their scars when heavy rain temporarily washes the rocks and vegetation free of salt. It is known that *Patella* spp. do browse upon damp rocks during periods of exposure (Orton, 1929; Stevenson, cited by Thorpe, 1956), especially at night, and this has also been observed at St Andrews. Browsing during the period of exposure is least important to those animals which inhabit the lower tide levels, most important to those near the upper limits of the shore. The increased responsiveness to splash and tolerance to diminished salt content of the water shown by limpets near high water mark (Arnold, 1957) can be readily interpreted as an adaptation to adverse environmental conditions which, together with increased radula length (Brian & Owen, 1952), enables these animals to maintain the minimum feeding period necessary for their growth and reproduction.

SUMMARY

Further experiments on the response of *Patella vulgata* to sea water and to the major ionic constituents of sea water are described. These responses cannot be attributed to recognition of variation in pH, osmotic pressure or minor ions. Instead they can be related to chloride ion concentration, modulated by the presence of calcium ions. The calcium ion concentration appears quite critical and the nature of the response given by the limpet alters when this is varied. It is suggested that these responses provide a means by which animals living on the higher portions of the shore can take advantage of spray or of periods of light rain or high humidity to prolong the feeding time into the periods of exposure by the tide.

REFERENCES

Arnold, D. C., 1957. The response of the limpet, *Patella vulgata* L., to waters of different salinities. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 121–8.

Brian, M. V. & Owen, G., 1952. The relation of the radula fraction to the environment in *Patella*. *J. Anim. Ecol.*, Vol. 21, pp. 241–9.

Croghan, P. C., 1958. The survival of *Artemia salina* (L.) in various media. *J. exp. Biol.*, Vol. 35, pp. 213–18.

Freeman, R. F. H. & Rigler, F. H., 1957. The response of *Scrobicularia plana* (da Costa) to osmotic pressure changes. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 553–67.

Harvey, H. W., 1955. *The Chemistry and Fertility of Sea Waters*. Cambridge University Press.

Kirk, P. L. & Moberg, E. G., 1933. Microdetermination of calcium in sea water. *Industr. Engng Chem. (Anal.)*, Vol. 5, pp. 95–8.

Orton, J. H., 1929. Observations on *Patella vulgata*. III. Habitat and habits. *J. mar. biol. Ass. U.K.*, Vol. 16, pp. 277–88.

Pantin, C. F. A., 1931. Adaptation of *Gunda ulvae* to salinity. *J. exp. Biol.*, Vol. 8, pp. 63–82.

—— 1946. *Notes on Microscopical Technique for Zoologists*. Cambridge University Press.

Ranade, M. R., 1957. Observations on the resistance of *Tigriopus fulvus* (Fischer) to changes in temperature and salinity. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 115–19.

Thorpe, W. H., 1956. *Learning and Instinct in Animals*. London: Methuen.

J. mar. biol. Ass. U.K. (1959) 38, 581–587
Printed in Great Britain

SOME PARAMETERS OF GROWTH IN THE COMMON INTERTIDAL BARNACLE, *BALANUS BALANOIDES* (L.)

By H. Barnes and Margaret Barnes
The Marine Station, Millport, Scotland

(Text-figs. 1–6)

It is difficult to determine the weight or nitrogen content of the living tissues of an operculate barnacle without destroying it. It has been customary, therefore, in ecological work to express growth in terms of various parameters determined from repeated measurements of the shell. Moore (1934) employed shell volume calculated from the height and basal diameters; others (Costlow & Bookhout, 1953, 1956; Mawatari, Hirosaki & Kobayashi, 1954*a*, *b*) have used the area of the basis. Most commonly, however, in growth-rate studies the length of the basis measured through the rostro-carinal axis has been used (Hatton, 1938; Barnes & Powell, 1953). Recently, working with animals cultured in the laboratory when the cast of an individual could be obtained subsequent to ecdysis, Costlow & Bookhout (1957) have used the size of the mouthparts as a measure of growth after first establishing their relation to body size. The space, both areal and volumetric, occupied by a sedentary animal is of primary importance in studies of its ecology and measurements of shell-size are, therefore, adequate for many purposes. For some aspects of growth and ecology it is, nevertheless, very desirable that the relation between such parameters and others, more directly connected with the living material, should be established.

THE MATERIALS AND METHODS

The specimens of *Balanus balanoides* (L.) were collected from near mid-tide level, individuals being selected that had had unrestricted growth and were, therefore, of the typical conical shape. Two collections were made; the first was in the autumn, just prior to the time at which fertilization takes place, and when the reproductive products were fully developed, and the second in the early winter after fertilization when the reproductive products were minimal. The shell dimensions were obtained using a travelling microscope. The body (prosoma and thorax) was then carefully separated, by cutting the attachment to the opercular valves, and weighed. Ovarian tissue when present was removed and weighed separately. After weighing, the body or ovary was transferred to a Kjeldahl flask, wet ashed in the usual way and the nitrogen determined as ammonia by a standard procedure for milligram quantities; the ammonia was absorbed in a saturated solution of boric acid and titrated with N/40 or N/80 hydrochloric acid. For the smallest animals pooled samples were used. Total shell weight and shell volume were determined on a separate series of individuals in which the animal's body was carefully removed without destroying the operculum. The shells were weighed dry and then carefully filled with plasticine and re-weighed; the shell volume (mantle cavity) was calculated from the increase in weight and the previously determined density of plasticine.

Wet weight of the body (W), wet weight of ovary (O), their total nitrogen contents (N_W and N_O), and the weight of calcareous shell (W_S) are expressed in mg, length of the rostro-carinal diameter (L) along the basis in mm, and volume of the mantle cavity (V) is given in μl.

RESULTS

Body weight and total nitrogen

The relation between body weight and its total nitrogen content is shown in Fig. 1. It is clear that the relation is linear and that the results fall on two separate lines which represent animals collected in autumn and winter. In the former the tissues include the fully developed male reproductive organs and their products which in the latter are only poorly developed. The regression of total nitrogen on body weight is

$$N_W = 0{\cdot}0086 + 0{\cdot}0215W$$

for the autumn animals, and

$$N_W = 0{\cdot}0530 + 0{\cdot}0143W$$

for the early winter animals. For a given body weight, above about 5 mg, which represents the approximate size at which the animals reach sexual maturity, the autumn animals have a higher nitrogen content than those collected in early winter, and this is consistent with the fact that a high nitrogen content (3·1 % wet weight) was found for the semen expressed from ripe animals.

Body weight and ovarian tissue

The female reproductive organs and their products are conspicuous in the base of the mantle cavity of animals collected in the autumn and may be easily separated from the body. Fig. 2 suggests that at smaller body weights the relation is not linear, the relative weight of female reproductive tissue falling off with decreasing body weight. When tested by the appropriate statistical technique the deviation from linearity is not, however, significant

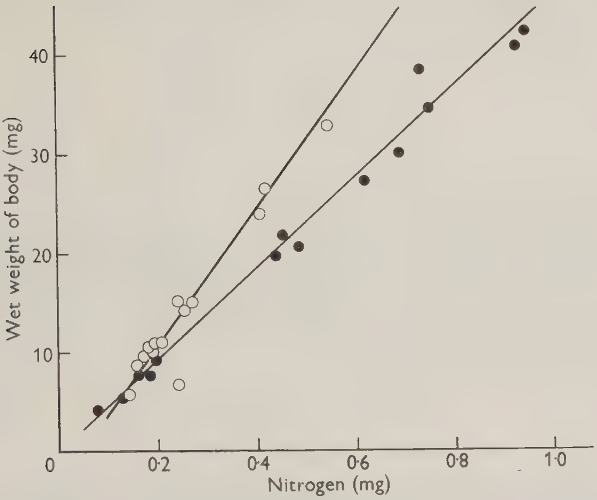


Fig. 1. *B. balanoides*: relation between body weight and total nitrogen content; —●—, animals taken in early autumn with well-developed male organs and reproductive products; —○—, animals taken in early winter with poorly developed male organs: regression lines drawn.

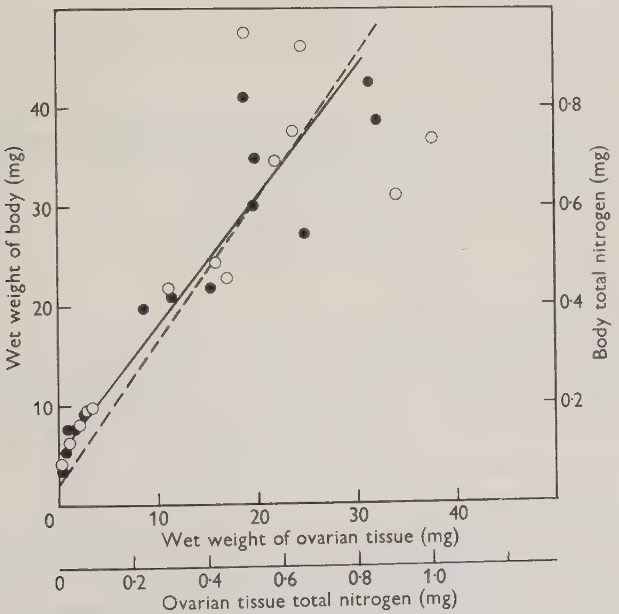


Fig. 2. *B. balanoides*: relation between body weight and ovarian tissue weight (—●—) and body total nitrogen and ovarian tissue total nitrogen (--○--): regression lines drawn.

and the relations may be expressed by the following linear regressions for the weight and nitrogen content

$$O = 0{\cdot}7684W - 3{\cdot}6282,$$

and

$$N_O = 0{\cdot}9168N_W - 0{\cdot}0342.$$

Shell length and body weight or nitrogen

It is evident from Figs. 3 and 4 that, whereas the relation between shell length and body weight (or total nitrogen) is linear in the autumn animals, this is not the case with the winter animals. Statistical examination indicates that there is no significant departure from linearity for the autumn animals; the linear regression equations are

$$W = 4{\cdot}4113L - 23{\cdot}3849,$$

and

$$N_W = 0{\cdot}0960L - 0{\cdot}5061.$$

The departure from linearity for the winter animals is significant and curvilinear regression equations were, therefore, determined. They are as follows:

$$W = -4{\cdot}6938L + 0{\cdot}3593L^2 + 22{\cdot}4883,$$

and

$$N_W = -0{\cdot}1047L + 0{\cdot}0069L^2 + 0{\cdot}5632.$$

Shell length and shell volume

The relation between these two parameters (Fig. 5) is not linear and the fact that transformation to logarithmic values fails to achieve linearity indicates that shell volume is not a simple power function of rostro-carinal diameter. A curvilinear regression was again fitted, giving

$$V = -26{\cdot}6192L + 2{\cdot}8188L^2 + 71{\cdot}8680.$$

Shell weight and shell volume

It is evident from Fig. 6 that, as would be expected, the weight of the calcareous shell is a linear function of its internal volume. The regression equation is

$$V = 11{\cdot}4080 + 0{\cdot}3077W_S.$$

The acquisition of material

In many places animals (*B. balanoides*) reach maturity during the first season's growth from a settlement in the spring; according to the conditions a size up to 15 mm (L) may be reached. Neglecting the material present in the original cyprid, the organic and inorganic matter acquired by an animal of any given size between settlement and the early autumn when the reproductive organs are fully ripe may be readily estimated from the preceding regression

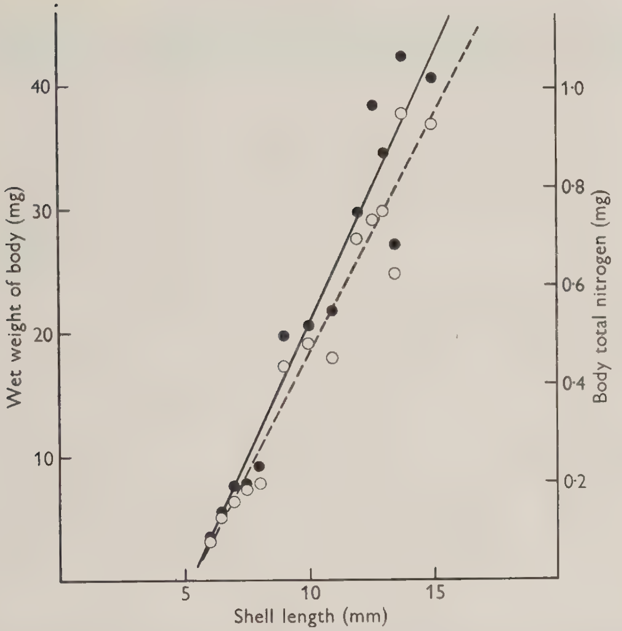


Fig. 3. *B. balanoides*: relation between body weight and shell length (—●—) and body total nitrogen and shell length (--○--) for animals taken in early autumn: regression lines drawn.

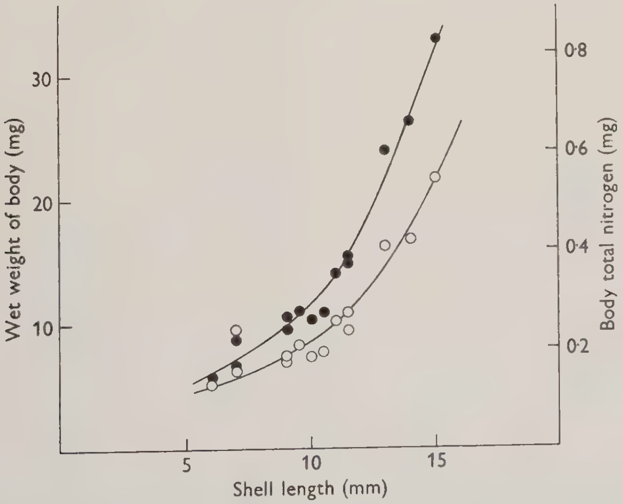


Fig. 4. *B. balanoides*: relation between body weight and shell length (—●—) and body total nitrogen and shell length (—○—) for animals taken in early winter: curvilinear regressions drawn.

equations. Figures are given for animals of 10 and 15 mm length in the following table, the values for the male reproductive tissue being calculated by subtraction from the fully ripe and spent regression equations:

Length (L)		10	15
Body	Weight (W)	20·7	42·8
	Nitrogen (N_W)	0·45	0·94
Male organs	Weight	9·2	9·9
	Nitrogen	0·24	0·39
Ovary	Weight (O)	8·1	29·3
	Nitrogen (N_O)	0·38	0·83
Shell		248	960

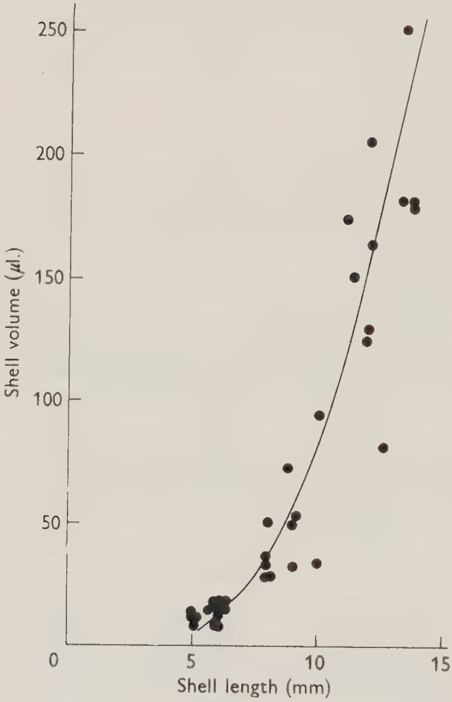


Fig. 5

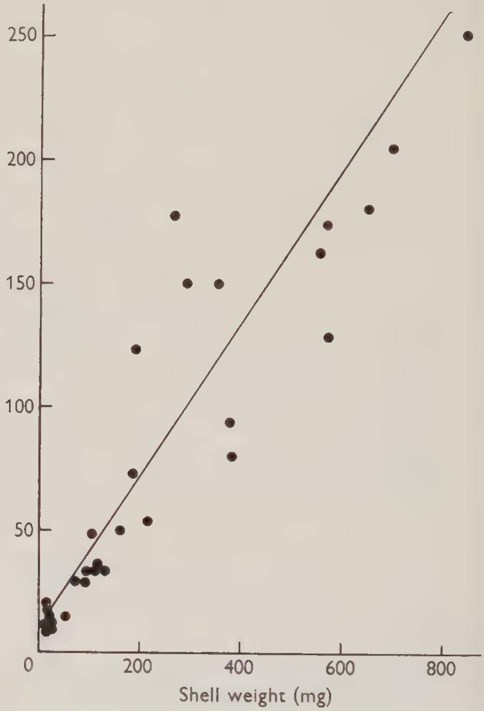


Fig. 6

Fig. 5. *B. balanoides*: relation between volume of mantle cavity and shell length: curvilinear regression drawn.

Fig. 6. *B. balanoides*: relation between volume of mantle cavity and shell weight: regression line drawn.

SUMMARY

The relation between body weight, weight of reproductive tissues, their nitrogen content, and shell length and volume, have been investigated for *Balanus balanoides*. The results are presented in graphs and as regression equations.

REFERENCES

BARNES, H. & POWELL, H. T., 1953. The growth rate of *Balanus balanoides* (L.) and *B. crenatus* Brug. under varying conditions of submersion. *J. mar. biol. Ass. U.K.*, Vol. 32, pp. 107–28.

COSTLOW, J. D. (Jr.) & C. G. BOOKHOUT, 1953. Molting and growth in *Balanus improvisus*. *Biol. Bull., Woods Hole.*, Vol. 105, pp. 420–33.

—— —— 1956. Molting and shell growth in *Balanus amphitrite niveus*. *Biol. Bull., Woods Hole*, Vol. 110, pp. 107–16.

—— —— 1957. Body growth versus shell growth in *Balanus improvisus*. *Biol. Bull., Woods Hole*, Vol. 113, pp. 224–32.

HATTON, H., 1938. Essais de bionomie explicative sur quelques espèces intercotidales d'algues et d'animaux. *Ann. Inst. océanogr. Monaco*, T. 17, pp. 241–348.

MAWATARI, S., HIROSAKI, Y. & KOBAYASHI, S., 1954*a*. Settlement and growth of acorn barnacle, *Balanus amphitrite communis* Darwin. I. *Misc. Rep. Res. Inst. natural Resour. Tokyo*, No. 33, pp. 46–55.

—— —— —— 1954*b*. Settlement and growth of acorn barnacle, *Balanus amphitrite communis* Darwin. II. *Misc. Rep. Res. Inst. natural Resour. Tokyo*, No. 34, pp. 48–57.

MOORE, H. B., 1934. The biology of *Balanus balanoides*. I. Growth rate and its relation to size, season and tidal level. *J. mar. biol. Ass. U.K.*, Vol. 19, pp. 851–68.

J. mar. biol. Ass. U.K. (1959) 38, 589–597
Printed in Great Britain

THE INFLUENCE OF TEMPERATURE ON THE REPRODUCTION AND MOULTING OF *LEPAS ANATIFERA* L. UNDER LABORATORY CONDITIONS

By Bhupendra Patel

Marine Biology Station, Menai Bridge (Anglesey)

(Text-figs. 1–2)

Lepas anatifera L. is generally found on floating objects in tropical and subtropical oceanic waters, where sea temperatures exceed 18–20° C.

A drifted buoy covered with recently settled and adult *Lepas anatifera* L., washed ashore near Cable Bay on the south-west of Anglesey, however, provided an opportunity to study in detail the processes of moulting and reproduction in this species under laboratory conditions.

Animals were carefully detached from the buoy and were kept in glass crystallizing dishes. Sea water was circulated through a long thin-walled coiled glass tube immersed in a tank controlled by a thermostat. A constant flow of warm water was thus maintained over the animals throughout the experiment. This was found necessary to keep them healthy and in normal condition; they rapidly became lethargic and moribund if the water was allowed to become stagnant.

The barnacles were fed on *Artemia* larvae and on a paste prepared from powdered mammalian liver. Small pieces of *Mytilus* tissue (1–2 mm) were also offered; these were rapidly grasped by the cirri and engulfed. Very large pieces (about 5 mm) were accepted, but the animals had difficulty in swallowing them (cf. Howard & Scott, 1959). However, uneaten *Mytilus* tissue and liver caused fouling of the water, so *Artemia* larvae were generally used and the animals seemed more healthy when fed on live food.

We found that in isolation the barnacles lived healthily with the stalks unattached. The stalks which had been carefully freed from their original attachment showed no evidence of refixing themselves, for example to the glass dish. However, when several such individuals were kept in the same dish, they were often found grasping and attempting to feed on each other's stalk, thus occasionally causing injury. The injured stalks decayed, followed often by the death of the animal. In one instance the stalk started to decay and a few days later cirral activity ceased and the animal appeared dead. When the stalk of the animal was lifted the thick outer integument separated, leaving the muscular part of the stalk attached to the animal. On the following day the fleshy stalk disappeared, the other barnacles in the dish having devoured it. The stalkless animal was then isolated; it resumed its normal activity and lived for a further 2 weeks. It is unlikely that this type of damage would arise if the animals were growing together attached to a common object, because then only the leathery outer surface of the stalk would be exposed to attack and the individuals would also be able to move away from each other by bending the stalk.

Colour of ovary

The normal colour of the ovary in *Lepas* is deep blue (Darwin, 1851; von Willemöes Suhm, 1876; Groom, 1894) as are the recently fertilized egg masses, though later the embryos turn to purple, red and pink as development proceeds. The original colour of the ovaries of the specimens washed ashore was blue, as also were the egg masses. On the Munsell Colour Chart they matched purple blue 5·0, value 4/chroma 6. However, after the animals had been kept in the laboratory and fed on *Artemia* larvae, the newly developed ovaries were seen to be distinctly pink (Munsell red 5·0, value 6/chroma 10) and the colour changed only slightly to a salmon peach tint (Munsell red 5·0, value 8/chroma 4) by the time the embryos were ready to hatch. A similar though less dramatic difference in colour of the ovary has been noticed also in operculate barnacles, such as *Balanus crenatus* Bruguière, *B. amphitrite* var. *denticulata* (Broch), *B. perforatus* Bruguière and *Chthamalus stellatus* Poli, when fed on *Artemia* in the laboratory, the ovaries being slightly more peach coloured in comparison with the yellow or orange ovaries of naturally fed specimens. The type of food thus plays an important role in determining the ovary colour.

In *Lepas anatifera* at least, this difference appears not to be due simply to an additional pigment derived from the *Artemia*, for there is no sign of the presence of any deep blue colour. The blue colour in *Lepas* eggs is considered to be an astaxanthin-protein complex (Ball, 1944), as also is the blue pigment of the oceanic Siphonophore, *Velella lata*, which Fox and Haxo (1958) believe to be derived from crustacean food. It seems probable therefore that an element in the oceanic plankton on which these animals feed contains an essential precursor of the blue astaxanthin-protein complex.

Reproduction

Like operculate Cirripedes and unlike a few pedunculate forms, *Lepas* is an hermaphrodite animal; it bears embryos four to five weeks after settlement (Skerman, 1958; Evans, 1958). Groom (1894) observed in detail the process of fertilization in *Lepas anatifera* L. and our observations confirm his. Occasionally some undeveloped and cytolysed eggs were observed in the egg mass; it was thought that these eggs may not have been fertilized. Failure to effect complete fertilization of the egg mass was considered not unlikely, because the animals, not being attached to their natural substratum, were unable to copulate and insert the penis normally in the mantle space of the adjacent specimens. Laboratory conditions might also have reduced the potency of spermatozoa. At every successful copulation the individual acting as female contracted its stalk as though to squeeze the ova through the oviducts and up into the mantle cavity where they became attached to the fraenae, the semi-circular folds of skin at the base of the mantle space (Darwin, 1851).

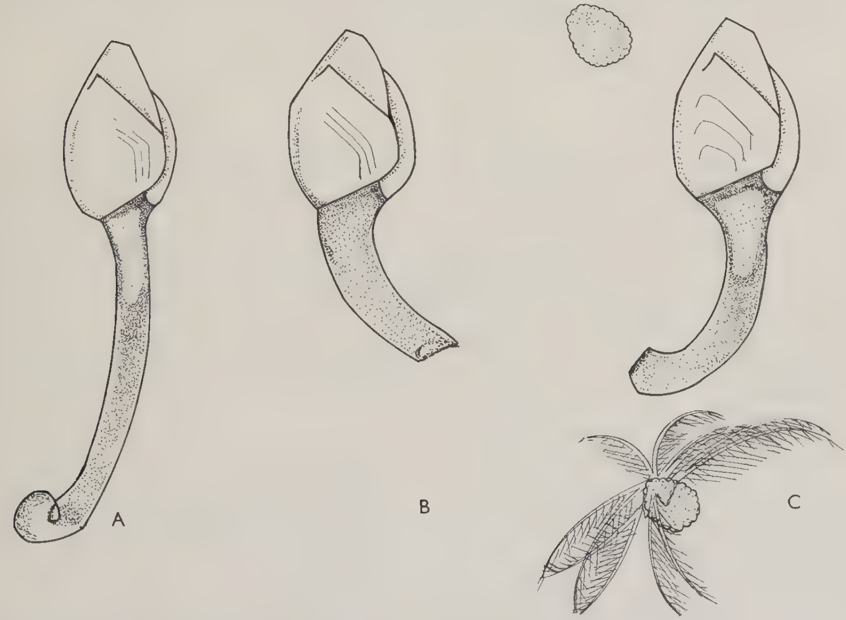


Fig. 1. A single individual of *Lepas anatifera* L. at different phases of breeding cycle. A, Before copulation. The large ovary is visible through the stalk. B, Just after copulation. The ovary has disappeared and the stalk is contracted. C, After liberation and moulting. The two egg lamillae are visible, one still attached to the cast skin. The ovary has redeveloped.

The ovary, originally visible through the stalk, was observed to have disappeared after copulation was completed. This fact could be used to ascertain when an animal had been fertilized. The clear stalk generally remained contracted for two to three days till a new ovary was formed and began to show through the stalk.

The sequence of changes visible outside the barnacle is presented in Fig. 1. One *Lepas*, which had a clearly defined pink ovary showing through the stalk, was marked and photographed (Fig. 1 A). It was then put into contact with other animals. Some time later, the marked individual was found in copula; after copulation the ovary disappeared and the stalk remained contracted (Fig. 1 B). The *Lepas* was then kept isolated and fed liberally. Three to four days later a new ovary appeared. On completion of the embryonic development (after seven days at 25° C) it liberated egg masses which were seen to be hatching as stage I nauplii and were accompanied by the cast skin. In Fig. 1C one of the egg masses is still attached to the cast skin. This sequence of events could be observed in most specimens. The changes in the appearance of the ovary as seen through the upper part of the stalk might usefully be employed in the field to determine whether an animal had recently been fertilized or whether it still carried a large unfertilized ovary, without the necessity of dissecting it, provided that the skin pigmentation at the junction of the stalk and shell was not too dense.

Self- or cross-fertilization

To investigate if *Lepas anatifera* L. is a self- or cross-fertilizing hermaphrodite, a number of specimens were isolated in glass crystallizing dishes, with a current of water passing through, and were fed liberally for a long time, daily observations being made to note if any nauplii or egg lamellae were liberated. The temperature of the water was maintained within the breeding range of the animal (see below). Unfortunately only very limited numbers of specimens were available for this investigation.

After several weeks these animals, which had shown no sign of breeding, were placed in groups of 4 to 5 animals in larger dishes and maintained under otherwise identical conditions.

During the period of isolation the animals developed new ovaries at all temperatures between 15 and 25° C, but penis activity was observed only between 19 and 25° C. Small amounts of sticky seminal fluid were often found smeared on the walls of the dish where such specimens were being kept. However, the animals failed to self-fertilize at any of the temperatures at which they were kept. They appeared to be obligatory cross-fertilizing hermaphrodites like *B. balanoides* L., *B. balanus* (= *porcatus*, da Costa) (Crisp, 1954; Barnes & Crisp, 1956) and *Elminius modestus* (Crisp, 1958). No sooner were they grouped than copulations were observed at temperatures between 19 and 25° C. Clearly under the conditions of these experiments self-fertilization does not occur, but cross-fertilization takes place readily (Table 1).

Breeding temperature

The results of a number of experiments in which groups of animals were maintained for several weeks at approximately constant temperatures are shown in Table 2. It will be noted that breeding was possible between 19 and 25° C but not at or below 15° C, nor at temperatures higher than 30° C. These results were confirmed by the disappearance of ovaries only from the barnacles in dishes kept at 19 and 25° C, and later by the presence of liberated nauplii only in the same dishes. Probably 16° C is close to the lower limit of the breeding range because Skerman (1958) found *L. anatifera* var. *testudinata* in New Zealand waters bearing embryos at temperatures varying from 17 to 20° C, while Darwin's records (1851), with one exception, are from areas where the temperatures generally exceed this range. Darwin includes a record from the Bass Straits, where the range lies between 13 and 17° C. Evans' (1958) observations during a voyage from Dakar to Barbados indicate that this species breeds readily between 24 and 26° C, and also agree with the behaviour of specimens in the laboratory.

TABLE 1. ABSENCE OF SELF-FERTILIZATION IN *LEPAS ANATIFERA* L. IN THE LABORATORY

(The temperature range of the experiment was 19–25° C)

Number of *Lepas* available during experiment	Condition of specimens	Total no. of barnacle days*	No. of liberations of nauplii seen
25	Isolated	667	0
31	Grouped	619	26

TABLE 2. INFLUENCE OF TEMPERATURE ON BREEDING ACTIVITY IN *L. ANATIFERA* L.

Temperature (°C)	No. of barnacles used	Total no. of barnacle days	No. of liberations observed	Embryonic period (days)	Size of ripe embryos
8–10	9	53	0	—	—
15–16	4	96	0	—	—
19–20	19	431	12	11–12	290 × 136μ
24–25	12	188	14	6–7	266 × 122μ
30–31	7	160	0	—	—
34–36	12	Died	—	—	—

* 'Barnacle days' = no. of specimens × no. of days under observation.

It is interesting to note that the breeding optimum, 19–25° C, is close to the optimum range of cirral activity as shown by Southward (1957), (Fig. 2). It is surprising that Boëtius (1952–3) found recently settled *Lepas* in Danish waters where, except in very shallow and locally warm pools, such temperatures would not be expected, though it is also possible that, if its normal planktonic food were available, breeding might occur over a wider temperature range than when fed on *Artemia*. However, the specimens kept in the laboratory at 15–16° C appeared healthy, developed large ovaries, and became fertilized within a few days of their temperature being raised to 24–25° C.

Rate of embryonic development

Groom (1894), following the work of von Willemöes Suhm (1876), studied all the stages of embryonic development, but did not mention the time required for complete development. The exact time required by a fertilized egg to reach its final stage capable of hatching as a stage I nauplius is important in an animal which breeds continuously, as this determines its fecundity

(Crisp & Davies, 1955). The time of the embryonic development was investigated as follows:

The fertilized animals were removed and kept isolated as soon as they were observed in copula or when their ovaries had disappeared from the stalk. They were kept under observation and examined every 6–12 h until liberation of egg masses or nauplii occurred. This was always coincident with moulting.

The time of the embryonic development varied with temperature as shown in Table 2.

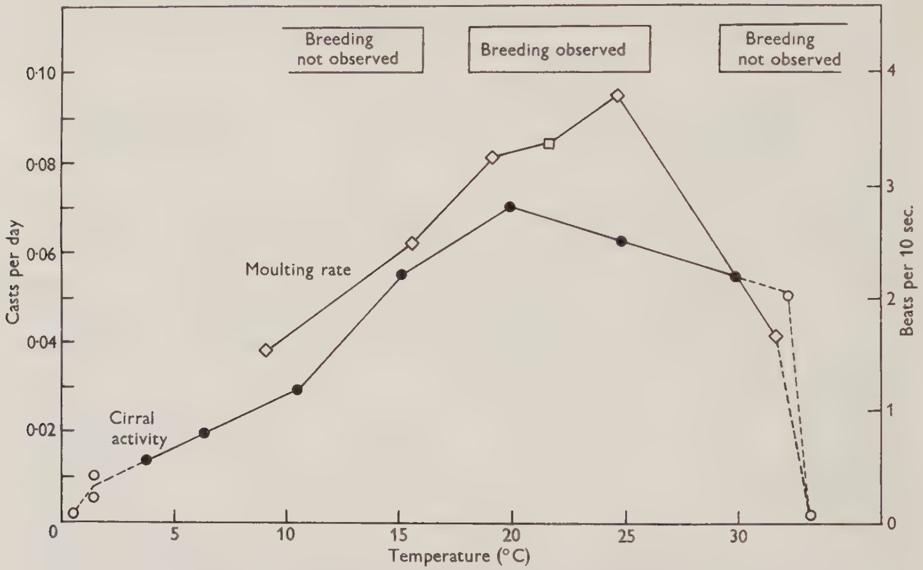


Fig. 2. The effect of temperature on cirral activity (after Southward, 1957), on moulting rhythm and on breeding activity in *Lepas anatifera* L.

The ripe embryos were measured and were found larger both in length and in breadth when the parents had been maintained at the lower temperature and smaller in size when the parent was kept at the higher temperature (Table 2). This phenomenon has been found also in other operculate barnacles. Groom (1894) measured the sizes of the eggs containing ripe embryos from *Lepas anatifera* L. grown under natural conditions, and found their length to be 250 μ. His measurement of the breadth of ripe embryos as stated seems absurdly small and is probably so due to a misprint; it should surely read 145 μ not 45 μ. His figures are then of the same order as ours. Evidently the eggs produced in the laboratory with abnormal food were of the normal size found in nature, as also were the stage I nauplii.

Ecdysis

Within the sub-class Cirripedia the mode of ecdysis differs between sessile and pedunculate forms, as Darwin (1851) noticed. In the sessile forms the chitinous layers of the animal body and also the inner lining of the operculum and the shell are moulted regularly. We confirmed that in *Lepas* the chitinous layers of the prosoma, cirri and penis, together with the lining of the oesophagus and rectum, were regularly cast, but on no occasion did the cast skin include the membrane lining the walls, nor was there any ecdysis of the integument of the stalk. On average an animal took about 15–30 min in completely relieving itself of the cast.

TABLE 3. EFFECT OF TEMPERATURE ON THE FREQUENCY OF MOULTING IN *LEPAS ANATIFERA* L.

	Isolated specimens				Grouped specimens				All specimens		
Temperature (°C)	No. of *Lepas*	Barnacle days	No. of casts	Inter-moult period (days)	No. of *Lepas*	Barnacle days	No. of casts	Inter-moult period (days)	Barnacle days	No. of casts	Moulting rate (cast/day/barnacle)
8–10	—	—	—	—	9	53	2	26·5	53	2	0·0380
15–16	29	1214	76	16·0	4	96	6	16·0	1310	82	0·0625
18–20	3	84	6	14·0	19	431	33	13·0	515	39	0·0760
21–22	11	363	30	12·0	—	—	—	—	363	30	0·0840
24–25	11	220	26	8·5	12	188	22	8·5	508	48	0·0950
30–31	3	9	—	—*	7	160	7	23·0	169	7	0·0415
	Starved specimens										
15–16	11	495	16	31·0	—	—	—	—	495	16	0·0320

* Only three *Lepas* were kept for about 3 days. None moulted during this period, hence no definite figure for the inter-moult period.

All except the last row were fed liberally on *Artemia* and dried liver.

During the experiments described in the section on reproduction observations were made on moulting. Table 3 shows the effect of temperature on the rate of moulting (Fig. 2). It will be seen that with the increase of temperature from 10 to 25° C, the rate of moulting increased in an approximately linear fashion, but at 30–31° C the rate of moulting decreased and the animals subsequently died at 34–35° C. It can be seen from Fig. 1 that the three activities, namely cirral activity (Southward, 1957), moulting rate and breeding increased regularly to an optimum at about 20–25° C, and thereafter decreased sharply.

A few animals were kept without food at 16° C and compared with animals fed on *Artemia* the moulting rate fell considerably (Table 3), cirral activity ceased in the starved individuals, and eventually many died. The fact that even at the lower part of its temperature range *Lepas* cannot survive long without food suggests that it is less able to withstand starvation than most sessile barnacles, since forms such as *Balanus balanoides* L. and *Chthamalus stellatus* Poli can be kept for many months in the laboratory without food.

Relation between moulting and reproduction

Crisp and Patel (1958) showed for the first time that there was generally a relationship between moulting and reproduction in sessile Cirripedes.

In *Lepas*, fertilized specimens in which the ovaries had disappeared from the stalk were placed each in a separate dish and examined daily. None of these individuals moulted whilst carrying embryos. After embryonic development was completed, they usually liberated nauplii and simultaneously underwent ecdysis. In a few instances the animals liberated first and moulted on the following day, but in most cases the salmon-peach-coloured egg masses containing unfertilized eggs with free stage I nauplii (and sometimes a few stage II nauplii) were given off at the same time as, or within an hour or so of, the act of moulting. In one instance only, a *Lepas* kept at 24–25° C moulted although bearing egg masses. This occurred eight days after the ovary had disappeared, by which time the eggs should have been hatching. At the end of another three days it gave off salmon-peach-coloured egg masses. On microscopical examination it was found that none of the eggs, though normally oviposited and cemented together, had developed, and they appeared to be unfertilized.

From Tables 2 and 3 it may be seen that the duration of embryonic development is on average slightly less at all temperatures than the inter-moult period of unfertilized specimens. Thus if the recently moulted individual is able to be fertilized immediately, there would be no interference between the moulting cycle and breeding cycle, the moult normally taking place simultaneously with, or soon after, the completion of embryonic development. On one occasion two animals were put together at 19–20° C, one of them moulted (it was marked) and after a few hours it was found receiving sperms. After the ovary had disappeared from the stalk this specimen was separated and it liberated nauplii as expected after 12 days and moulted on the 13th day.

I am greatly indebted to Dr D. J. Crisp for his generous help and guidance in the experimental work and in the preparation of the manuscript.

SUMMARY

The moulting and breeding activities of *Lepas anatifera* L. were studied under laboratory conditions.

When removed from the substratum and fed on *Artemia* and powdered mammalian liver the animals remained healthy and resumed their normal activities if kept in constantly changing sea water. If the animals were not fed or water ceased to circulate they became lethargic and slowly died.

The ova, normally of a blue colour, develop instead to a pink when the animals are fed on *Artemia* larvae.

Isolated specimens showed penis activity but did not self-fertilize; when

grouped together they became fertilized and produced viable nauplii at temperatures between 19 and 25° C. After successful copulation the acting female shed the ova by contraction of the stalk into the mantle space, and a new ovary developed after 4–5 days.

The rate of moulting increased with rise in temperature from 10 to 25° C but fell after further rise in temperature, 34–36° C being rapidly lethal.

Gravid *Lepas* did not moult while they were carrying embryos, and the liberation of nauplii was always accompanied or followed shortly by ecdysis.

REFERENCES

Ball, E. G., 1944. A blue Chromoprotein found in the eggs of the Goose barnacle. *J. biol. Chem.*, Vol. 152, pp. 627–34.

Barnes, H. & Crisp, D. J., 1956. Evidence of self-fertilization in certain species of barnacles. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 631–9.

Boëtius, J., 1952–3. Some notes on the relation to the substratum of *Lepas anatifera* L. and *Lepas fascicularis* E. & S. *Oikos*, Vol. 4 (2), pp. 112–17.

Crisp, D. J., 1954. The breeding of *Balanus porcatus* (da Costa) in the Irish Sea. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 473–496.

—— 1958. The spread of *Elminius modestus* Darwin in North-west Europe. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 483–520.

Crisp, D. J. & Davies, P. A., 1955. Observations *in vivo* on the breeding of *Elminius modestus* grown on glass slides. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 357–80.

Crisp, D. J. & Patel, B. S., 1958. Relation between breeding and ecdysis in Cirripedes. *Nature, Lond.*, Vol. 181, pp. 1078–79.

Darwin, C., 1851. *A Monograph on the sub-Class Cirripedia. The Lepadidae or Pedunculate Cirripedes.* 400 pp. London: Ray Soc.

Evans, F., 1958. Growth and maturity of the barnacles *Lepas hillii* and *Lepas anatifera*. *Nature, Lond.*, Vol. 182, pp. 1245–46.

Fox, D. L., 1953. *Animal Bichromes and Structural Colours*, 379 pp. Cambridge University Press.

Fox, D. L. & Haxo, F. T., 1958. Pigments and algal commensalism in the blue oceanic Siphonophore *Velella lata*. *Abst. XV Int. Congr. Zoology.*

Groom, T. T., 1894. On the early development of Cirripedia. *Phil. Trans.* B, Vol. 185, pp. 119–232.

Howard, G. K. & Scott, H. C., 1959. Predaceous feeding in two common gooseneck barnacles. *Science*, Vol. 129, pp. 317–18.

Skerman, T. M., 1958. Rates of growth in two species of *Lepas* (Cirripedia). *N.Z. J. Sc.*, Vol. 1 (3), pp. 402–11.

Southward, A. J., 1957. On the behaviour of barnacles. III. Further observations on the influence of temperature and age on cirral activity. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 323–34.

Von Willemöes Suhm, 1876. Development of *Lepas fascicularis* and the 'Archizoea' of Cirripedia. *Phil. Trans.* B, Vol. 166 (1), pp. 131–54.

J. mar. biol. Ass. U.K. (1959) 38, 599–604
Printed in Great Britain

A NOTE ON SOME PHYSICAL CONDITIONS FOR CULTIVATING *OXYRRHIS MARINA*

By M. R. Droop

Marine Station, Millport, Scotland

(Text-figs. 1–4)

This note concerns a series of experiments to determine the best conditions of salinity, temperature and pH for cultivating the euryhaline phagotrophic dinoflagellate *Oxyrrhis marina* Dujardin.

The strain of *Oxyrrhis* employed was isolated from a brackish pool at Tvärminne, Finland (Droop, 1953*a*, *b*). The culture medium for the experiments contained soil extract and an artificial sea water, SW 1 (NaCl, $MgCl_26H_2O$, KCl, and $CaSO_42H_2O$ in the proportions by weight 15:2·5:0·4:0·5), and for food a small quantity of the yeast *Saccharomyces exiguus* was administered daily from an agar culture with a wire loop.

The rate of cell division during the logarithmic phase of growth is a measure of the suitability of conditions prevailing. Since growth is by binary fission the number of divisions per day is conveniently given by the relative growth rate when expressed as the binary logarithm of the relative increase in cell numbers per day. This parameter, denoted by k, is simply the slope of the growth curve when cell numbers are expressed as binary logarithms.

Cell counts were made in a deep chamber which allowed the whole of a 0·1 ml. sample to be counted if required. Five samples were usually counted but when numbers exceeded 100 per sample it was more convenient to count several areas within the sample with the aid of a squared eyepiece graticule and compute accordingly.

The statistical treatment in the salinity experiments followed conventional procedures of regression analysis (Snedecor, 1946) and was carried out on the transformed counts. I am indebted to my colleague T. B. Bagenal for advice and for undertaking the analysis.

SALINITY

Different salinities were obtained by varying the amount of SW 1 in the medium. Cultures of *Oxyrrhis* can thrive if the salinity lies between 4‰ and 130‰.[1] Greater salinities were not tested, but below 4‰ cultures failed.

The salinity experiments were required to determine the optimum salinity and also the effect of transfer from one salinity to another. They consisted of two sets of cultures grown at room temperatures and initial pH 7·4 in the following salinities: 4, 8, 16, 32 and 64‰, from inocula adapted to 8‰ in the one set and 64‰ in the other. The parameters to be determined were, relative growth rate, 'apparent initial viable count', and 'initial total count'.

---

[1] ‰, titrated chloride expressed as g NaCl per l.

TABLE 1. GROWTH OF *OXYRRHIS* IN CULTURES OF DIFFERENT SALINITY AND INOCULA FROM TWO DIFFERENT SOURCES

(Counts expressed as $\log_2$ cells per ml. Those shown in italics lie off the logarithmic phase of growth and were not used in calculating the regression coefficients.)

Culture salinity	Inoculum from 8‰ culture					Inoculum from 64‰ culture				
	4‰	8‰	16‰	32‰	64‰	4‰	8‰	16‰	32‰	64‰
3rd day	4·9	6·75	8·41	5·32	—	6·64	8·99	10·00	8·82	8·02
	4·32	6·58	8·45	5·64	—	7·90	9·09	10·57	8·32	7·96
	5·32	6·58	8·49	6·32	—	7·84	8·87	10·26	8·53	7·13
	3·32	5·58	8·64	6·13	—	7·02	9·60	9·64	8·82	7·71
	4·9	6·75	8·12	4·32	—	7·49	6·27	9·22	8·90	7·64
5th day	6·13	9·46	12·84	9·86	—	8·36	10·92	13·32	12·32	9·10
	5·90	9·70	12·23	9·88	4·32	8·45	12·71	13·58	12·00	10·07
	6·49	9·66	12·02	9·80	4·32	8·32	11·23	13·45	10·57	9·34
	6·13	9·45	11·32	9·94	3·32	8·22	12·50	12·81	11·82	9·45
	6·32	9·71	11·49	9·88	3·32	8·17	11·91	13·32	11·32	9·34
7th day	5·90	12·57	16·52	11·74	5·32	*8·41*	14·91	16·02	13·58	10·14
	5·64	12·82	16·48	12·57	5·32	*8·12*	15·00	16·21	13·90	10·54
	6·49	13·11	16·63	12·23	5·64	*7·96*	15·58	16·39	14·17	9·64
	6·64	13·12	16·22	13·18	5·32	*8·07*	15·52	16·47	13·58	10·26
	6·9	12·78	16·71	11·49	5·64	*7·64*	15·58	16·08	13·90	10·38
10th day	*6·79*	*15·32*	*16·28*	*15·16*	—	—	*14·96*	*15·94*	*15·12*	12·52
	7·13	*15·46*	*16·28*	*15·58*	8·18	—	*14·70*	*16·92*	*15·64*	12·21
	5·32	*15·80*	*16·68*	*15·75*	8·23	—	*15·00*	*15·83*	*15·12*	12·32
	6·91	*15·52*	*16·86*	*15·58*	6·49	—	*15·09*	*15·96*	*15·64*	13·21
	7·23	*15·52*	*16·52*	*15·64*	—	—	*15·25*	*15·49*	*15·78*	12·75
12th day	—	*16·52*	—	*16·52*	—	—	—	—	—	—
	—	*16·64*	—	*16·04*	10·85	—	—	—	—	—
	—	*16·55*	—	*16·20*	10·81	—	—	—	—	—
	—	*16·46*	—	*16·58*	10·93	—	—	—	—	—
	—	*16·35*	—	*16·24*	—	—	—	—	—	—
14th day	—	—	—	—	12·32	—	—	—	—	—
	—	—	—	—	12·13	—	—	—	—	—
	—	—	—	—	12·77	—	—	—	—	—
	—	—	—	—	11·09	—	—	—	—	—
	—	—	—	—	11·09	—	—	—	—	—

The ten growth curves are shown in Table 1. The earlier counts of each cover the logarithmic phase of growth, and are, therefore, fitted statistically by the general equation

$$n = kt + c,$$

when n represents $\log_2$ cells per ml. at time t, k and c being constants, the former the relative growth rate, the latter the logarithm of the 'apparent initial viable count', i.e. n at $t = 0$. The two constants and their 95% fiducial limits for the ten regressions are plotted against salinity in Figs. 1 and 2.

Since a clear maximum for k occurred at 16‰ in both experiments (Fig. 1) it can be concluded that the optimum salinity at 16‰ was not influenced by the salinity of the inoculum.

In contrast to k, the second constant c did depend to some extent on the source of the inoculum. The value of c must be determined primarily by the 'initial total count', that is the number of cells introduced, but it would be influenced either by mortality of cells on transfer or by an initial lag preceding the start of regular cell division. Either effect could result from a change in the composition of the medium. They are not distinguished from each other in the regressions and are conveniently treated as wholly mortality.

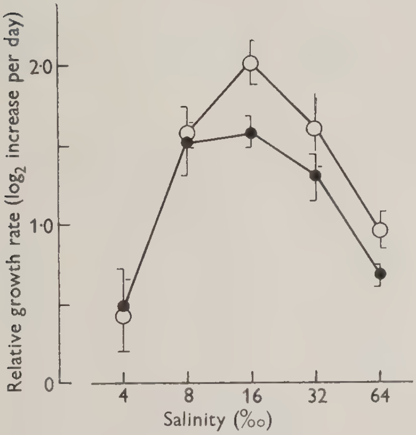


Fig. 1

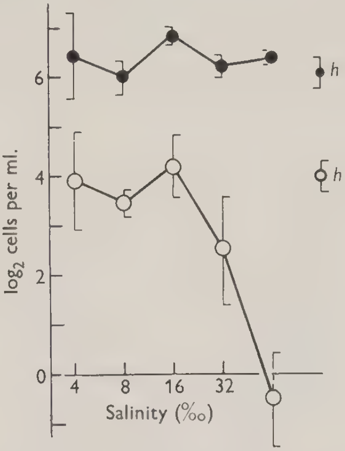


Fig. 2

Fig. 1. Relative growth rate (k) as a function of salinity. Open circles, 8‰ adapted inoculum; filled circles, 64‰ adapted inoculum. 95% fiducial limits indicated.

Fig. 2. Log 'apparent initial viable count' (c) as a function of salinity. Open circles, 8‰ adapted inoculum; filled circles, 64‰ adapted inoculum. Log 'initial total count' (h) in each case is shown on the right. 95% fiducial limits indicated.

The 'initial total count' was obtained by counting sample 0·06 ml. aliquots from the inoculum cultures and dividing by 6 (since an inoculum of 0·06 ml. was being given to 6·0 ml. of culture medium). h, the logarithm of this, in the 8‰ experiment was 3·95 (fiducial limits ±0·30), and in the 64‰ experiment 6·08 (limits ±0·31). Fig. 2 shows that h is significantly higher than c only in the case of the two cultures of highest salinity in the 8‰ experiment, from which the conclusion is drawn that, while the transfer from a high to low salinity was tolerated without shock, the more extreme cases of reverse transfer were not experienced without harm to the population.

Microscopic observation of the behaviour of cells on transfer confirmed this conclusion and, moreover, showed that the correct interpretation of the phenomenon was mortality on transfer and not initial lag. Thus, cells suddenly transferred from 8 to 64‰ quickly became lean and angular and apparently dehydrated, shedding their flagella in most cases and all but 2 or 3% failing to recover; whereas they became swollen and almost completely spherical and sluggish on transfer in the downward direction and they did not shed their flagella but regained shape and activity within the hour.

TEMPERATURE AND pH

A medium of 16‰ salinity was used in the temperature and pH experiments. Initial pH in the former was 7·4 and temperature in the latter 22·5°. The temperatures between 22·5° and 34° were obtained with a conventional incubator and those between 19° and 6° with a refrigerated water bath. Initial pH was adjusted with NaOH or HCl and read with B.D.H. capillator outfit at the time of inoculation and again after 6 days.

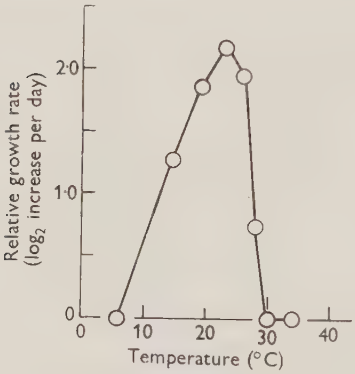


Fig. 3

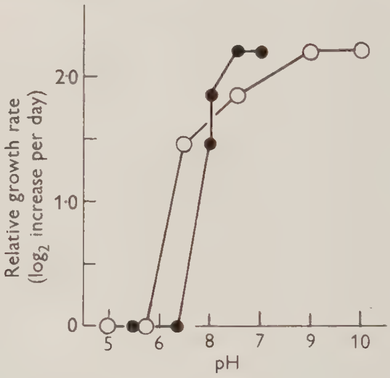


Fig. 4

Fig. 3. Relative growth rate (k) as a function of temperature.

Fig. 4. Relative growth rate (k) as a function of pH. pH measured: open circles, at start; filled circles, after 6 days.

The results of the two experiments were not subjected to statistical analysis, since the sole parameter required was relative growth rate. They are shown in Figs. 3 and 4, where relative growth rate is graphed against temperature or pH. There was a temperature optimum of 22°–23°, an upper tolerance limit, of 28°, and a Q_{10} of about 2·7 between 10° and 20°. A pH of 7 or over appears to be suitable.

DISCUSSION

A maximum division rate of 2·2 per day is higher than is normally met among dinoflagellates and may be correlated with *Oxyrrhis*'s phagotrophic habits. Braarud (1951) obtained maxima of 0·32 for *Exuviaella baltica*, 0·55 for

Amphidinium sp., 0·95 for *Peridinium trochoideum*; while Braarud & Rossavik (1951) obtained 0·55 for *Prorocentrum micans*, Braarud & Pappas (1951) 0·89 for *Peridinium triquetrum*, and Sweeney (1954) 0·67 for *Gymnodinium splendens*.

Oxyrrhis is most often encountered in brackish rock pools above the high-water mark and sometimes occurs in water of very high salinity. At the other extreme, it is seldom found in pools whose salinity is lower than 4‰, in which respect it differs from many other supra-littoral species which tolerate or even prefer the lower salinities (Droop, 1953*a*, table 10; 1955, table 1). The optimum salinity for the neritic species studied by Braarud (1951) ranged from 16 to 20‰ with upper and lower toleration limits only slightly narrower than those reported here for *Oxyrrhis*. It seems that salinity tolerance *per se* cannot satisfactorily account for the absence of neritic species from supra-littoral pools or of *Oxyrrhis* from the sea.

The response to sudden changes in salinity is interesting and shows *Oxyrrhis* well fitted to its habitat, for the salinity of sea-water marks the limit to which a pool can suddenly be raised by sea splash, any further rise being necessarily by evaporation and therefore slow. In south Finland, where this strain originated, this figure is 6‰. On the other hand, the reverse change, which is brought about by flooding with rain water, can be quite rapid, especially if mixing is not delayed. It has been observed previously (Hopkins, 1938) that the physiological response to changes in salinity of marine Protozoa not possessing a rigid periplast or contractile vacuoles is to contract or to swell. This is due to a temporary unbalance between the osmotic pressure of the internal and external media. Recovery is stated to be due to adjustment by the passage of salts through the membrane, though it might equally be brought about by mobilization or immobilization of carbohydrate reserves of high osmotic pressure. *Oxyrrhis* certainly has great powers of adjustment, particularly when the change is made gradually. The fact that dehydration is a greater hazard than bursting speaks for the tensile strength of the periplast.

The response to pH was as expected, for a pH 8–9 is normal for a pool containing *Oxyrrhis* and one below 7 is seldom encountered in the aerobic layers of supra-littoral pools. The temperature curve is conventional: its peak suggests that *Oxyrrhis* is a summer organism, which indeed it is, though the high rainfall in this country or ice cover in Finland would in any case keep the pools empty of flagellates during the winter months. The upper laboratory temperature limit of 28° is often exceeded for short periods in pools containing large populations of *Oxyrrhis* during the summer; for instance, I obtained midday records of 30° in Finland (Droop, 1953*a*). Possibly other more southern races of this species tolerate higher continuous temperatures.

SUMMARY

Maximum division rate of the dinoflagellate *Oxyrrhis marina* Dujardin feeding on *Saccharomyces exiguus* occurred in cultures of salinity 16‰, temperature 22·5° and pH 8–10, and was 2·2 per day.

Salinities below 4‰, pH below 6·5 and continuous temperatures over 28° were not tolerated.

Sudden extreme changes in salinity were tolerated in the downward but not in the upward direction.

REFERENCES

Braarud, T., 1951. Salinity as an ecological factor in marine phytoplankton. *Physiol. Plant.*, Vol. 4, pp. 28–34.

Braarud, T. & Pappas, I., 1951. Experimental studies on the dinoflagellate *Peridinium triquetrum* (Ehrb.) Lebour. *Avh. norske VidenskAkad.*, 1957, No. 2, pp. 1–23.

Braarud, T. & Rossavik, E., 1951. Observations on the marine dinoflagellate *Prorocentrum micans* Ehrb. in culture. *Avh. norske VidenskAkad.*, 1951, No. 1, pp. 1–18.

Droop, M. R., 1953*a*. On the ecology of flagellates from some brackish and fresh-water rock-pools of Finland. *Acta bot. fenn.*, Vol. 51, pp. 1–52.

—— 1953*b*. Phagotrophy in *Oxyrrhis marina* Dujardin. *Nature, Lond.*, Vol. 172, p. 250.

—— 1955. Some new supra-littoral Protista. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 233–45.

Hopkins, D. L., 1938. Adjustment of the marine amoeba, *Flabellula mira* Schaeffer to changes in the total salt concentration of the outside medium. *Biol. Bull., Woods Hole*, Vol. 75, p. 337.

Snedecor, G. W., 1946. *Statistical methods.* 4th Edition. Iowa: State College Press.

Sweeney, B. M., 1954. *Gymnodinium splendens*, a marine dinoflagellate requiring vitamin B_{12}. *Amer. J. Bot.*, Vol. 41, pp. 821–24.

J. mar. biol. Ass. U.K. (1959) 38, 605–620
Printed in Great Britain

WATER-SOLUBLE FACTORS IN THE NUTRITION OF *OXYRRHIS MARINA*

By M. R. Droop

The Marine Station, Millport, Scotland

(Text-figs. 1–8)

Oxyrrhis marina Dujardin is a non-photosynthetic dinoflagellate whose obvious mode of nutrition is phagotrophy. The method of feeding was described by Barker (1935). It is an extremely euryhaline and hardy organism and is apparently very successful in brackish habitats such as supralittoral rock pools and ditches (Droop, 1953*a*). Owing to the ease with which it can be cultivated *Oxyrrhis* is a choice for the initial study of phagotrophy among the predominantly plant-like Dinoflagellata.

Axenic cultures (i.e. cultures free from other organisms of any kind) are required for nutritional studies, but they are not often easily established when the subject is phagotrophic. *Oxyrrhis* was no exception, despite the ease with which it can be kept in the laboratory. Bacteria-free cultures of a Finnish race were established in 1951 (Droop, 1953*b*), but there then appeared to be a requirement for a living food organism such as a yeast or alga. Dr J. J. A. McLaughlin (Haskins Laboratories, New York, personal communication) succeeded, however, with axenic cultures of another strain in 1954, but unfortunately was not in a position to pursue the matter.

Dr McLaughlin's media contained, in addition to sources of water-soluble nutrients, such substances as soya meal, corpus luteum extract, cream, and beef serum in an attempt to meet the suspected fat requirement. These ingredients proved difficult to handle and unreliable in Dr McLaughlin's experience and, moreover, in my hands they entirely failed to support axenic growth. The first reliably successful axenic cultures at Millport were obtained in 1955 with a medium supplemented with 4 ml./l. of neutralized, strained but unfiltered, lemon juice. At a later date the juice was replaced by a carbon tetrachloride extract of the rind, which has the advantage of being composed entirely of fat-solubles. Isolation and identification of this fat-soluble 'lemon factor' is not completed, and will not be discussed further in this paper, which is concerned with water-soluble components of the medium.

MATERIALS AND METHODS

The strain of *Oxyrrhis* referred to as the Finnish race (Millport No. 18; Culture Collection of Algae and Protozoa, the Botany School, Cambridge, No. LB 1133/1) and used in these experiments was isolated by myself from a supra-littoral pool at Tvärminne, Finland, in 1951. To obtain axenic cultures, monoxenic ones, in which the food organism was an obligate phototroph (*Nannochloris oculata*), were simply incubated in the dark with lemon juice. Tests showed these cultures to be free of algae after two transfers.

Stocks were maintained in medium E 6 (Provasoli, McLaughlin & Droop, 1957) supplemented with the lemon factor.[1] The extract referred to as 'LF' henceforth is a Sohxlet carbon tetrachloride extract of autoclaved, then oven-dried (70° C) lemon rind, concentrated to 10 ml. per lemon, and kept well-stoppered in complete darkness. LF was shaken up with the medium beforehand and the solvent driven off on autoclaving, leaving the extractives in fine suspension. Alternatively, in later experiments not concerned with carbon nutrition, LF was administered from ethanolic solution, which obviated shaking.

Glassware was cleaned with 'Lux' then chrome-sulphuric acid followed by repeated rinsing in tap, then distilled, water. Synthetic media were prepared from AR quality chemicals with glass-distilled water. Approximately 0·6 ml. of inoculum was used to start experimental cultures of 6 ml. capacity in 15 × 150 mm Pyrex test-tubes plugged with cotton-wool (aluminium caps in some experiments as a check against chemical contamination from cotton-wool). Cultures were incubated in darkness at 22° C. Elimination of carry-over from the complex to simpler media in the experiments was effected by serial transfers, ten being regarded as sufficient for this purpose. A bacteriological peptone or medium E 6 was used for frequent routine sterility tests, a necessary precaution since phagotrophy can obscure bacterial contamination in cultures. Cell counts were made in a chamber 0·67 mm deep with the aid of a squared eyepiece graticule, and are expressed as cells per mm^3 to the nearest whole number.

DEVELOPMENT OF A DEFINED BASAL MEDIUM

The first step in simplifying the stock medium E 6 was to replace the liver extract and Bacto Tryptone by 3 mg/l. tryptophane, 400 mg/l. Bacto Vitamin-free Casamino Acids (VFC) and 3 ml./l. of the B vitamin mixtures, D 7, 8 and 9 (Table 1). Yield of cultures then increased to over 100 cells per mm^3 and was maintained thus over many transfers.

The next stage was to replace VFC by synthetic mixtures of amino-acids (AA I–AA V, Table 1) with, however, only limited success, as the amino-acids gave a reduced yield which was restored by 200 mg/l. VFC. It later transpired that the new medium lacked metabolizable carbon.

---

[1] 1 l. of supplemented E6 contains: Oxo Liver Infusion (L25), 250 mg; Bacto Tryptone, 250 mg; glucose, 250 mg; soil extract, 12·5 mg; KNO_3, 50 mg; K_2HPO_4, 5 mg; $MgSO_4 7H_2O$, 5 mg; LF, 3 ml.; natural sea water, 500 ml.

Soil extract could be omitted and artificial solutions SW 1 and SW 2 (Table 6; Provasoli *et al.*, 1957) and a dispersing agent for LF, sodium taurocholate, could substitute for the natural sea water in E 6, provided the liver infusion was kept. But with liver infusion and Tryptone replaced as above neither natural sea water nor soil extract were replaceable by the buffered trace metal solutions usually used for this purpose (e.g. TM2 and the buffer tris(hydroxymethyl)aminomethane (TRIS) as used in S36 and S46, etc. (Provasoli *et al.*, 1957).

An alternative to the obvious hypothesis that unknown growth factors were being supplied by both soil extract and natural sea water was that the mixtures replacing them were toxic. Both TRIS and EDTA (ethylenediaminetetraacetic acid, the chelating agent in TM2) were known to be toxic to some organisms, and the possibility that TM2 was unbalanced could not be ruled out. So indeed it proved, for eventually soil extract and natural sea water were successfully replaced by a synthetic medium containing neither TRIS nor EDTA.

TABLE 1. S49, PARTIALLY DEFINED 'UNTAILORED' MEDIUM
(LF being the only undefined component)

SW 1*	250 ml.
SW 2*	5 ml.
TM 11B*	10 ml.
Glycylglycine	500 mg
L-Histidine	200 mg
K_2HPO_4	10 mg
KNO_3	100 mg
Na taurocholate	3·0 mg
LF	3·0 ml.
Fourteen supplementary solutions†	each 4·0 ml.
H_2O to	1·0 l.
pH adjusted to 8·0 before autoclaving	

* Cation mixtures, see Table 6.

† Supplementary mixtures (amounts unless otherwise stated in mg per l.):

D7: putrecine, 100; spermine, 100; choline, 500.

D8: niacin, 100; Ca pantothenate, 100; pyridoxine, 20; riboflavin, 5·0; *p*-aminobenzoic acid, 10; inositol, 1000.

D9: thiamine, 200; biotin, 0·5; vitamin B_{12}, 0·05; folic acid, 1·0; folinic acid, 0·2; thioctic acid, 0·5.

PP1: guanine, 1000; adenine, 500; uracil, 300; thymine, 300; cytidylic acid, 300.

AA I: DL-alanine, 10,000; DL-aspartic acid, 10,000; glutamic acid, 10,000; glycine, 10,000.

AA II: L-arginine, 10,000; L-histidine, 5000; DL-lysine, 10,000.

AA III: DL-isoleucine, 1000; DL-phenylalanine, 1000; DL-leucine, 1000; L-tyrosine, 1000.

AA IV: DL-methionine, 1000; DL-threonine, 2000; DL-tryptophane, 1000.

AA V: DL-serine, 2000; L-proline, 1000; L-valine, 1000.

FA I: Na acetate (anhyd.), 10,000; butyric acid, 1·0 ml.; propionic acid, 1·0 ml.; valeric acid, 1·0 ml.

FA II: succinic acid, 10,000; α-ketoglutaric acid, 10,000; fumaric acid, 10,000; malic acid, 10,000.

FA III: pyruvic acid, 10 ml.; lactic acid, 10 ml.; citric acid, 10,000.

CHHO I: arabinose, 10,000; rhamnose, 10,000; xylose, 10,000; glucose, 10,000; fructose, 10,000; galactose, 10,000; mannose, 10,000.

CHHO II: lactose, 20,000; sucrose, 20,000; maltose, 20,000.

In this, glycylglycine acted as pH buffer, and glycine or histidine as chelating agent in a new trace metal mixture (10 ml. of which contained such amounts of the metals as might occur in 1 g of algal protoplasm). The new medium retained the three B vitamin mixtures, the five amino-acid mixtures, SW 1, SW 2, potassium nitrate and phosphate, LF, and sodium taurocholate, but glucose was replaced by five solutions embracing a total of 22 carbon compounds.

The quantity of chelator was varied (glycine: 2, 10 and 100 mg/l.; histidine: 4, 20 and 200 mg/l.), but after two transfers it was clear that the two lower values of either amino-acid were unsuitable.

A medium with high histidine, S 49, was therefore adopted; it is given in full in Table 1. This was the first essentially synthetic basal medium to allow more than a couple of transfers. In its 'untailored' state it gave smaller yields than the original E 6, but in subsequent transfers, as the essential constituents were identified and brought up to adequate concentration, the yield came to surpass that of the original medium and no component of the latter effected any improvement, although mature cultures lasted rather longer in E 6.

Simplification of this appallingly complex medium was in principle a relatively straightforward undertaking, although it took many months, the work involved being comparatively great.

Carbon source

Identification of essential carbon sources involved a basal medium lacking the five carbon solutions, FA I–III and CHHO I and II. This medium supported a negligible amount of growth and, moreover, was scarcely improved by the single addition of any one of the carbon solutions (Table 2). But when the solutions were added in pairs (Table 3) FA III was shown to be essential and to give best results in combination with FA I, though it was not clear at this stage whether the other solutions also contained available compounds. A breakdown of FA III (Table 4) showed citrate to be the active component of this solution. The active component of FA I was similarly identified as acetate. Citrate, together with a high concentration of acetate, allowed good repeatable growth.

Subsequently no other compound included in solutions FA I–III and CHHO I–II proved to be available as a source of carbon, and the following were also ineffective: glycerol, erithritol, mannitol, sorbitol, dulcitol, raffinose, melizitose, glycogen, starch, inulin, dextrin, salicin and aesculin. Unavailable also was the carbon skeleton of any amino acid included in solutions AA I–V, or of asparagine. Ethanol, however, was utilized and was not toxic at a concentration of 4 ml./l. In contrast, propionic, butyric, valeric and lactic acids were toxic in concentrations over 0·04 ml./l.

By way of confirmation, yield in response to graded doses of sodium acetate, glucose, citric acid, ethanol and valine is shown in Fig. 1. The inoculum for these cultures had been carried for four transfers in medium S 49. Citrate thus proved not to be available as a bulk carbon source, whereas acetate served just this purpose since yield appeared as directly proportional to sodium acetate concentrations up to 0·2%. The requirement for citrate is discussed later.

TABLE 2. EFFECT OF SINGLE CARBON SOLUTIONS

(Basal medium: S 49 with the five carbon solutions omitted. Cells per mm^3 after 3 weeks' growth.)

No addition	FA I	FA II	FA III	CHHO I	CHHO II	All five solutions
			First transfer			
11	9	9	18	9	8	120
			Second transfer			
4	2	2	6	5	5	37

TABLE 3. EFFECT OF CARBON SOLUTIONS IN PAIRS

(Basal medium: S 49 with the five carbon solutions omitted. First transfer. Cells per mm^3 after 3 weeks' growth.)

No addition	FA I and FA II	FA I and FA III	FA I and CHHO I	FA I and CHHO II	FA II and FA III
9	10	122	9	9	19

FA II and CHHO I	FA II and CHHO II	FA III and CHHO I	FA III and CHHO II	CHHO I and CHHO II
9	8	22	28	8

TABLE 4. BREAKDOWN OF FA III

(Basal medium: S 49 with FA III omitted. Cells per mm^3 after 3 weeks' growth.)

No addition	Pyruvate	Lactate	Citrate	Pyruvate and lactate	Pyruvate and citrate	Lactate and citrate	Pyruvate and lactate and citrate
			First transfer				
11	9	8	89	9	82	69	64
			Second transfer				
6	5	0	32	3	42	20	26

Nitrogen source

When amino-acid solutions AA I–V were omitted, medium S 49 did not support more than a few cells per mm^3 in the first and later transfers. Furthermore, potassium nitrate had no effect on the medium either in the presence of the amino-acids or in their absence and it was subsequently omitted from all media. Oxidized nitrogen is evidently unavailable.

The single addition of AA I or especially AA V restored the depleted medium, whereas AA II–IV were without effect. A breakdown of AA I and V showed valine, proline and alanine as active, and aspartic and glutamic acids, glycine and serine as inactive. In later experiments valine proved the most readily available of the three amino-acids and yielded the best dose/response curves, although activity of the other two was of the same order (Fig. 2). No response was obtained to the ammonium ion, which was apparently too toxic at pH 8 to be of any use, nor to urea, uric acid or asparagine.

Valine was therefore adopted as N source, but a little proline was also retained, as it appeared to be stimulatory in early phases of cultures.

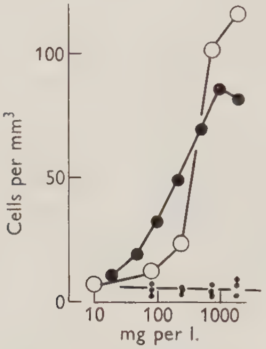


Fig. 1

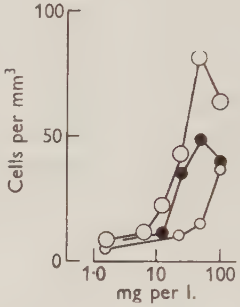


Fig. 2

Fig. 1. Carbon sources. Yield in response to increasing amounts of: sodium acetate (open circles); ethanol (filled circles); glucose, citric acid and valine (dots).

Fig. 2. Nitrogen sources. Yield in response to increasing amounts of: valine (large open circles; proline (filled circles); alanine (small open circles).

Growth factors

In the untailored medium S 49 possible growth factors were being supplied in solutions PP 1, D 7, D 8 and D 9. A conventional process of elimination proved D 9 to be the only essential mixture. Similarly, in D 9, thiamine, vitamin B_{12} and biotin proved essential and folic, folinic and thioctic acids unessential. In these analyses it was, of course, necessary to resort to serial transfers, owing to the minuteness of the requirements. The requirements for thiamine and vitamin B_{12} were absolute, that for biotin possibly not so (Table 5 and Fig. 7).

Dose/response curves for thiamine and vitamin B_{12} are given in Fig. 3. The thiamine requirement was previously reported in this *Journal* (Droop, 1958); thiazole replaces thiamine completely. Specificity towards the vitamin B_{12} analogues proved to be of the '*Ochromonas*' pattern (Kon, 1955) with the exception that factor A, a natural analogue containing 2-methyl-adenine in place of 4, 6-dimethyl-benzimidazole, showed slight activity in my experiments.

On the other hand, pseudo-vitamin B_{12}, the analogue containing adenine in the nucleotide, was quite inactive. A similar specificity has been reported by McLaughlin & Provasoli (1957) for two photosynthetic species of *Amphidinium*.

TABLE 5. REQUIREMENT FOR VITAMIN B_{12} DEMONSTRATED BY SERIAL TRANSFERS

(Dose level: 100 mµg/l. Yield in cells per mm^3)

No addition	With vitamin B_{12}	With factor A	With pseudo-vitamin B_{12}	With factor B
		First tranfer		
14	269	—	—	—
		Second transfer		
1	41	—	—	—
		Second transfer (repeat)		
1	133	11	1	1

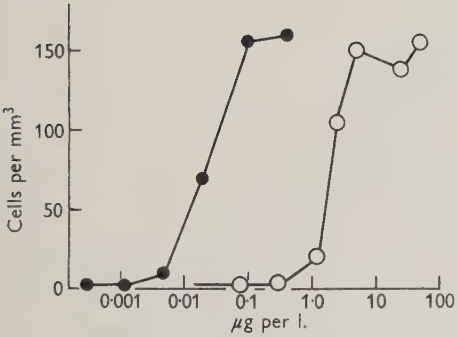


Fig. 3

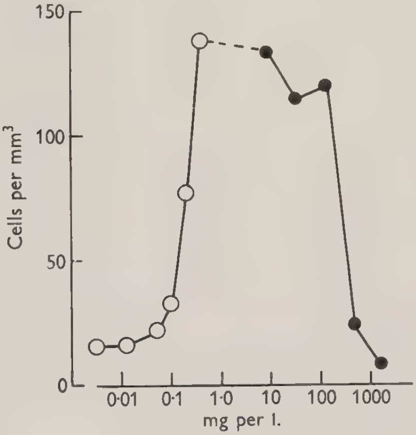


Fig. 4

Fig. 3. Response to increasing amounts of: thiamine (open circles); vitamin B_{12} (filled circles).

Fig. 4. Response to increasing amounts of citric acid (two experiments).

Citric acid

It was established that citrate, though apparently required, was not available as a bulk carbon source. It might, therefore, be functioning either as a growth factor or as a chelating agent.

Response to graded doses of citrate is shown in Fig. 4. The basal medium contained histidine and the metals of TM 11 B, but citrate was omitted. The inoculum had passed two passages in citrate-free medium. The response is seen to be sensitive to citric acid concentrations between 10 and 100 µg/l., while concentrations over 100 mg/l. were toxic. One would suppose that such a high order of activity would rule out chelation. However, in another experiment with a medium containing 50 mg/l. EDTA there was no response to citrate at the low end of the scale but growth was uniformly depressed, which suggested that chelation was, after all, involved.

A further set of experiments, undertaken with a basal medium lacking both citrate and histidine but containing the trace metals of TM 11 B, not only showed that it was possible to dispense with citrate if the correct amount of EDTA were present but also that glycine, citrate and EDTA all interacted in such a way as to suggest that their chelating properties were additive (Fig. 5). Thus, while best growth was obtained with 6·0 mg/l. EDTA in the absence of citrate, in its presence (40 mg/l. citric acid) only 1·0 mg/l. EDTA was required. Furthermore, with citrate and glycine both in the medium no EDTA was required.

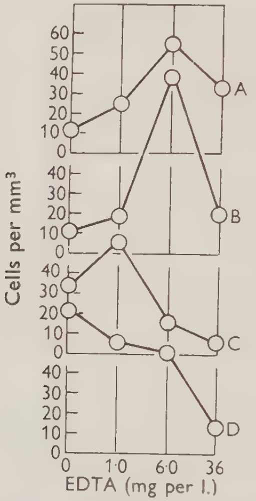


Fig. 5. Interaction of chelating agents: response to increasing amounts of EDTA. A, with no other chelating agent; B, with 200 mg/l. glycine; C, with 40 mg l. citric acid; D, with 200 mg/l. glycine and 40 mg/l. citric acid. Yield in cells per mm³, exploded ordinate, successive curves being displaced 50 units.

The apparent requirement for citrate is thus seen to be due to inadequate chelation by glycine or histidine. The fact that the ferric ion forms no complex with α-amino acids (Albert, 1950) should have made this conclusion obvious; indeed, in the absence of both citrate and EDTA a precipitate, which could be iron hydroxide, forms in the medium upon autoclaving.

On the other hand, the reason for poor growth in media with high (20–60 mg/l.) EDTA is not at all clear. Over-chelation is ruled out because calculation[1] shows the concentrations of the free ions of each of the metals in the mixture actually to be lower with the citrate-glycine or histidine mixtures than with even the highest concentrations of EDTA used. EDTA may indeed be toxic to *Oxyrrhis* for some entirely unconnected reason.

---

[1] By a method suggested by Spencer (1958), with the aid of published stability constants.

It is possible, however, to conclude empirically, that in a half-strength artificial sea-water medium with cationic concentrations as shown in Table 6, and in which the strongest chelators otherwise are valine and glycylglycine, the main function of chelation can be discharged by a variety of combinations of EDTA, citrate and glycine or histidine, bearing in mind that glycine and histidine do not chelate trivalent iron and that EDTA behaves as if it were inherently toxic.

pH *control*

The use of synthetic media introduces an urgent need for pH control, for a great many variables are influenced by pH. In particular, the degree of ionization and penetration of weak acids, bases and amphoteric substances is affected and consequently their utilization or toxicity. pH also enters as a term in the mass-action equations controlling the behaviour of heavy metals with chelating agents and on that account may not be ignored. It can be said with truth that a synthetic culture medium must be developed in relation to, and may only be useful over, a limited pH range.

pH control during growth does not present great problems except in certain circumstances. But the act of autoclaving a culture medium does create difficulties. Permanent damage may be done to a medium in which pH buffering is left to the carbonate-bicarbonate system, because the great rise in pH occasioned by the loss of carbon dioxide causes uncontrolled, usually irreversible, precipitation of various components. However, the practice of incorporating artificial buffers overcomes this difficulty to a large extent (Provasoli *et al.* 1957).

Since one seeks to prevent pH *rise* during autoclaving, the region where high buffer capacity is needed is immediately and for some distance above the setting. Thus, for a setting of pH 8, media buffered with glycylglycine ($pK_2 = 8{\cdot}1$)[1] should be improved by the presence of compounds having higher pK values. Valine ($pK_2 = 9{\cdot}6$), histidine ($pK_2 = 9{\cdot}7$) or glycine ($pK_2 = 9{\cdot}7$) serve this purpose in addition to their other functions (Fig. 6).

There are, however, valid objections to the use of organic acids in culture media on the grounds that they complicate the situation needlessly. Some pH buffers are also chelating agents; many of them (e.g. glycylglycine) are weak and can be ignored in adequately balanced media, but others have considerable influence and must be taken into account, as has been done in the case of histidine and glycine in the previous section. It is also undesirable that substances introduced for their physical effects should be metabolized, and due account should be taken of this possibility in any instance. But the converse is equally true: the physical properties of compounds introduced as nutrients cannot always be ignored.

---

[1] The relatively more toxic TRIS buffers in the same region.

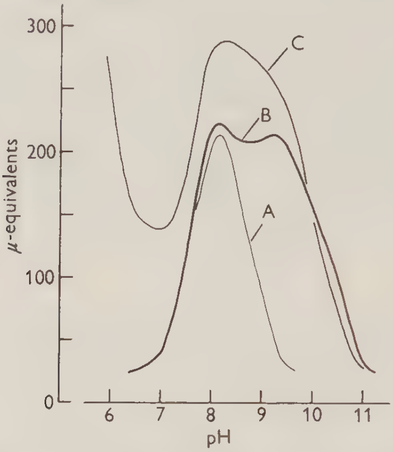


Fig. 6. Buffer capacity of some culture media in μ-equivalents of acid or alkali required to displace pH by 0·1. A, media with 3·8 mM glycylglycine or TRIS alone (e.g. S36, Provasoli *et al.* 1957); B, media with 3·8 mM glycylglycine and 3·3 mM glycine (e.g. S50, Droop, 1958); C, media with 3·8 mM glycylglycine, 1·3 mM histidine, 2·1 mM valine and 25 mM acetic acid (S69, Table 6). (Based on data from the '*Bufferule*' by the California Corporation for Biochemical Research.)

FINAL MEDIUM

The conclusions expressed in the previous pages are embodied in the two 'final' media S68 and S69 set out and annotated in Table 6. If handled carefully *Oxyrrhis* maintains heavy yields on continued subculturing in S69 (Fig. 7); consequently it is likely that all *absolute* water-soluble requirements are now defined.[1]

In Fig. 8 a typical growth curve in S69 and one in OX7 (a maintenance medium[2]) are contrasted with growth on living *Saccharomyces exiguus* (Droop, 1959). The contrast is sufficiently striking and is in a sense a measure of failure to substitute a non-living for a living diet. In this connexion it should be mentioned that although whole milk, egg yolk and lipids such as olive, linseed and palm oils can be ingested none have been found which improve the rate of growth or replace acetic acid. It would appear to be a living diet and not phagotrophy *per se* which makes for the high growth rate (Droop, 1953*b*).

Two further characteristics of axenic cultures which have been responsible for many delays and frustrations in this investigation are the inability of isolated cells to multiply, even in media which support normal subculturing indefinitely, and the inability of a population whose growth has been arrested by nutritional depletion to pick up again on being transferred to a complete medium. The rate of growth once depressed may stay so over many transfers, or even indefinitely. As can be imagined, these characteristics render the serial subculturing technique extremely difficult to handle. I am of the opinion that the explanation of this behaviour will prove to lie with the lipid factor and not the water-soluble nutrients, but one cannot be sure. However, no aqueous extract or hydrolysate of natural materials yet tried has been of any avail; and filter-sterilizing crude media to avoid destroying heat-labile substances has likewise been useless.

---

[1] Proline and histidine have now also been rigorously eliminated from the list of possible absolute requirements.

[2] One litre of OX7, which is now recommended for maintenance of axenic stocks, contains: Bacto Casitone, 125 mg; soil extract, 30 mg; Na acetate, 2 g; valine, 250 mg; D9 vitamins, 20 ml.; K_2HPO_4, 10 mg; LF, 3 ml.; natural sea water, 500 ml.

TABLE 6. MEDIA S68 AND S69

(Amounts per l.; pH adjusted to 8·0 before autoclaving. Note: glycine can replace L-histidine in S69.)

$NaCl$	15 g	Major ions of sea water (i.e. 250 ml. of 'SW 1')
KCl	400 mg	
$MgCl_2 6H_2O$	2·5 g	
$CaSO_4 2H_2O$	500 mg	
$SrCl_2 6H_2O$	6·5 mg	Minor constituents (i.e. 5·0 ml. of 'SW 2'. This can very likely be omitted)
KBr	33 mg	
$AlCl_3 6H_2O$	250 μg	
$RbCl$	100 μg	
KI	50 μg	
$FeSO_4 7H_2O$	2·5 mg	Trace metals (i.e. 10 ml. of TM 11 B)
$MnSO_4 H_2O$	100 μg	
$ZnSO_4 7H_2O$	100 μg	
$CuSO_4 5H_2O$	20 μg	
$CoSO_4 7H_2O$	2·5 μg	
$NaMoO_4 2H_2O$	1·2 μg	
EDTA*	6·0 mg	Chelating agent in medium S 68
Citric acid†	40 mg	Chelating agent in medium S 69
L-Histidine†	200 mg	Chelating agent and pH buffer at 9·2 in medium S 69
Glycylglycine	500 mg	pH buffer at 8·1
L-Valine	250 mg	N source and pH buffer at 9·6
K_2HPO_4	10 mg	P source
Na acetate (anhydr.)	2·0 g	C source
L-Proline	40 mg	Water-soluble growth factors
Thiamine	100 μg	
Biotin	50 μg	
Vitamin B_{12}	200 mμg	
Na taurocholate	3·0 mg	Dispersing agent for LF
LF	3·0 ml.	CCl_4 extract of lemon rind, containing an unknown lipid factor

* Omitted from S 69. † Omitted from S 68.

NUTRITIONAL STATUS

Dinophyceae have since Pascher (1914) and Fritsch (1935) been generally recognized as one of the great algal series; albeit one represented almost entirely by motile unicells.[1] They are not alone among algae in having phagotrophic representatives; this character they share with Euglenophyceae and Chrysophyceae. Starch staining blue with iodine is among the reserve products, though in my experience in many species, including *Oxyrrhis marina*, starch is only found in the cysts. Lwoff (1944, p. 215) and Hutner & Provasoli (1955, p. 19), however, stress the animal or protozoan tendencies shown by dinoflagellates.

---

[1] The possible affinity of *O. marina* with the Cryptophyceae (Dragesco, 1952) emphasizes Pascher's views regarding the near relationship between the two series.

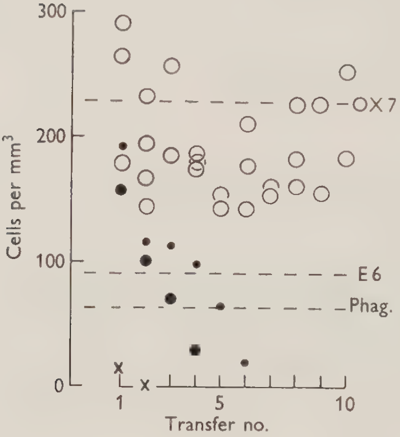


Fig. 7

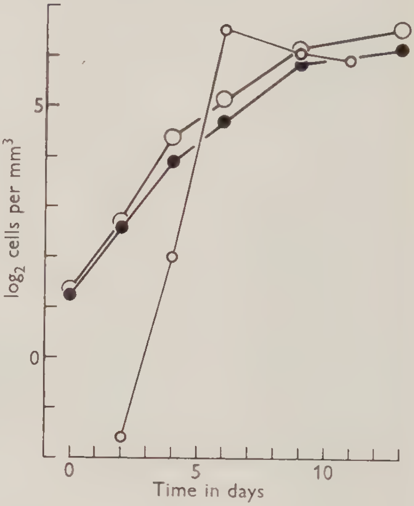


Fig. 8

Fig. 7. Yield in successive transfers in S 69 (open circles); S 69 without biotin (filled circles) (two experiments); S 69 without either thiamine or vitamin B_{12} (crosses). Yields normally obtained in undefined media indicated by broken lines: axenic cultures in OX 7 and E 6; monoxenic cultures on *Saccharomyces exiguus* in Erdschreiber ('phag').

Fig. 8. Typical growth curves: axenic cultures in OX 7 (large open circles) and S 69, 10th transfer (filled circles); monoxenic cultures on *Saccharomyces exiguus* in Erdschreiber (small open circles).

Superficially *Oxyrrhis* has an entirely animal-like nutrition, for it can rely, and probably does so in nature, on phagotrophy for all its major and accessory nutrients. Only when phagotrophy is denied it and an analysis then made of its nutritional requirements and abilities does a more plant-like nature emerge.

Taking carbon nutrition first: *Oxyrrhis* proves to be an 'acetate flagellate' (Pringsheim, 1935; Pringsheim & Hovasse, 1948; Hutner & Provasoli, 1951) since it uses acetic acid and ethanol, not glucose nor the carbon of amino-acids, for growth. Lwoff (1944) termed this kind of nutrition 'oxytrophy' in contrast to the 'haplotrophy' of organisms that use a wider range of substrates.[1] Lwoff's designations 'chlorophyte', 'leucophyte', 'protozoon', broadly correspond to the respective nutritional categories phototroph, oxytroph, haplotroph. However, a great many chlorophytes are oxytrophic in the dark while many others, including isolated tissues of phanerogams, utilize carbohydrate as well and to that extent may be regarded as being haplotrophic (Provasoli, 1938; Algeus, 1946; White, 1951; Pringsheim, 1952; Lewin, 1953). Although the number of possible substrates is even greater in animals such as ciliates, insects and vertebrates, so it is also in some fungi and bacteria (Kidder & Dewey, 1951; Trager, 1953; Albritton, 1954; Foster, 1949; Stephenson, 1949).

---

[1] These are subdivisions of what Lwoff called 'allotrophy', but now more generally known as chemotrophy.

Acetate organisms are thus characterized by limitations which they share with neither animals nor higher plants. They would not, therefore, appear to be in direct evolutionary line with plants or animals, which are probably derived from more versatile ancestors. The limitation according to current views (Lwoff, 1951) is probably one of phosphorylating enzymes rather than membrane permeability. Limited permeability is implied, however, in the resistance shown by many acetate flagellates to high concentrations of such penetrating and toxic substrates as acetic acid and ethanol, or, for that matter, to their typically highly polluted habitat (Hutner & Provasoli, 1951).

As regards nitrogen nutrition: although ability to employ nitrate as sole nitrogen source is confined to the plant kingdom, loss of the ability is found in some members of most phyla of plants. *Oxyrrhis* shares this state of slight but obligate heterotrophy (mesotrophy) with such photosynthetic algae as *Hemiselmis virescens* (Cryptophyceae) and *Chlamydomonas pulsatilla* (Chlorophyceae). Here nitrogen requirements are met by the ammonium ion or, failing that, any one of a number of simple organic compounds provided they can be made to yield an amino group. In fungi and bacteria greater and varying degrees of heterotrophy are to be found; they are exemplified by *specific* requirements for one or more amino-acids (Snell, 1951). But it is in animals (ciliates, insects, vertebrates) that nitrogen heterotrophy has proceeded farthest and become so stereotyped that it is possible to speak of the 'ten amino acids essential for protein synthesis' with little ambiguity (Kidder & Dewey, 1951; Trager, 1953; Rose, 1938). Against this background the mere inability to reduce nitrate would seem not to remove *Oxyrrhis* very far from the higher green plant.

Oxyrrhis requires but thiamine, vitamin B_{12} and biotin among B vitamins, thus showing a self-sufficiency toward the remainder that is typical of auxotrophic lower algae (Droop, 1957; Provasoli, 1958). Provasoli (1957) regards auxotrophy as indicating 'animality' ('vegetality' being typified by the need for plant hormones). Certainly, the lower members of the plant kingdom are likely to be more animal-like than the higher ones. On the other hand, many isolated phanerogamic tissues are also auxotrophic; fungi are commonly so. Even a vitamin B_{12} requirement is now recorded in a fungus (Adair & Vishniac, 1958) and in tumour tissues of spruce (Reinert & White, 1956). Furthermore, the pattern of specificity towards the vitamin B_{12} analogues typical of vertebrates and, by inference therefore, the most animal-like of the three patterns, is also shown by all the Chlorophyceae requiring vitamin B_{12} which I have examined.[1]

One is struck by the simplicity of the growth requirements in *Oxyrrhis*. But for the 'lemon factor' which in truth has been responsible for past failures with this organism, it is a typical acetate flagellate with an acetate flagellate's requirements and abilities, no more and no less plant-like. Phagotrophy thus emerges as an anomalous element in a conventional nutritional pattern. A progression *phototroph–oxytroph–phagotroph* could now be put beside the more usual (though equally theoretical) sequence *phototroph–haplotroph–phagotroph*. Such a progression might be characteristic of dinoflagellates and euglenids, but our knowledge is scanty. Although oxytrophy is well documented in the latter, the principal substrate for phagotrophic members is unknown (Storm & Hutner, 1953); and the only other non-photosynthetic dinoflagellate being studied is *Gyrodinium cohnii* for which there are as yet no published data.[2]

Phagotrophy appears to be commoner than saprotrophy in colourless dinoflagellates; photosynthetic phagotrophs are also reported. This might mean that in this alliance phagotrophy usually arises before the loss of photosynthetic pigments. It is also likely to have preceded the advent of any lipid requirements, which would otherwise be greatly disadvantageous to a free-living aquatic organism. Conversely, such requirements would survive without hindrance in established phagotrophs, with the result that their lipid metabolism would tend in the long run to become atrophied. On the other hand, organisms as large as *Oxyrrhis* have relatively limited cell surface, and are likely to find phagotrophy the most efficient method of feeding—particularly in nutritionally depleted environments. Isolation has many advantages; its disadvantages are thus avoided. An acetate organism which is also a phagotroph gets indeed the best of both worlds.

SUMMARY

The phagotrophic dinoflagellate *Oxyrrhis marina* can be cultivated indefinitely in the absence of other organisms of all kinds provided it is supplied with certain vegetable lipids.

Development of culture media in which all the water-soluble components are defined enabled the water-soluble nutrient and absolute growth factor requirements to be ascertained. The media were equivalent to a half-strength sea water buffered with glycylglycine and histidine with a trace metal mixture chelated with histidine and citrate.

---

[1] *Balticola droebakensis*, *B. buetschlii*, *Brachiomonas submarina*, *Chlamydomonas pulsatilla*, *Platymonas tetrathele* and *Stephanosphaera pluvialis*.

[2] Dr L. Provasoli, however, tells me that glucose is utilized by *G. cohnii*.

Acetate or ethanol (not any carbohydrate nor the carbon of amino-acids) serve as carbon source, and alanine, proline or especially valine (not NO_3^-, NH_4^+ or urea or other amino-acids) as nitrogen source. Auxotrophic requirements are met by the thiazole moiety of thiamine, vitamin B_{12} (near '*Ochromonas*' specificity), and biotin.

The nutritional status of *Oxyrrhis marina* is discussed.

REFERENCES

ADAIR, E. J. & VISHNIAC, H. S., 1958. Marine fungus requiring Vitamin B_{12}. *Science*, Vol. 127, pp. 147–8.

ALBERT, A., 1950. Quantitative studies of the avidity of naturally occurring substances for trace metals. I. Amino-acids having only two ionizing groups. *Biochem. J.*, Vol. 47, pp. 531–8.

ALBRITTON, E. C., 1954. *Standard Values in Nutrition and Metabolism.* 380 pp. Philadelphia: W. B. Saunders Co.

ALGEUS, S., 1946. Untersuchungen über die Ernährungsphysiologie der Chlorophyceen. *Bot. Notiser*, 1946, pp. 1–278.

BARKER, H. A., 1935. The culture and physiology of the marine dinoflagellates. *Arch. Mikrobiol.*, Vol. 6, pp. 157–81.

DRAGESCO, J., 1952. Le flagellé *Oxyrrhis marina*: cytologie, trichocysts, position systématique. *Bull. micrposcop. App.*, Ser. 2, Vol. 2, pp. 148–57.

DROOP, M. R., 1953*a*. On the ecology of flagellates from some brackish and fresh-water rockpools of Finland. *Acta bot. fenn.*, Vol. 51, pp. 1–52.

—— 1953*b*. Phagotrophy in *Oxyrrhis marina* Dujardin. *Nature, Lond.*, Vol. 172, p. 250.

—— 1957. Auxotrophy and organic compounds in the nutrition of marine phytoplankton. *J. gen. Microbiol.*, Vol. 16, pp. 286–93.

—— 1958. Requirement for thiamine among some marine and supra-littoral protista. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 323–9.

—— 1959. A note on some physical conditions for cultivating *Oxyrrhis marina*. *J. mar. biol. Ass. U.K.*, Vol. 38, pp. 599–604.

FOSTER, J. W., 1949. *Chemical Activities of Fungi.* 648 pp. New York: Academic Press Inc.

FRITSCH, F. E., 1935. *The Structure and Reproduction of the Algae.* Vol. 1, 791 pp. Cambridge University Press.

HUTNER, S. H. & PROVASOLI, L., 1951. The phytoflagellates. In *Biochemistry and Physiology of Protozoa*, Vol. 1, pp. 27–128. Ed. A. Lwoff. New York: Academic Press Inc.

—— —— 1955. Comparative biochemistry of flagellates. In *Biochemistry and Physiology of Protozoa*, Vol. 2, pp. 17–44. Ed. S. H. Hutner and A. Lwoff. New York: Academic Press Inc.

KIDDER, W. G. & DEWEY, V. C., 1951. The biochemistry of ciliates in pure culture. In *Biochemistry and Physiology of Protozoa*, Vol. 1, pp. 323–400. Ed. A. Lwoff. New York: Academic Press Inc.

KON, S. K., 1955. Other factors related to vitamin B_{12}. *Symp. biochem. Soc.*, No. 13, pp. 17–35.

LEWIN, J. C., 1953. Heterotrophy in diatoms. *J. gen. Microbiol.*, Vol. 9, pp. 305–13.

LWOFF, A., 1944. *L'évolution physiologique.* 308 pp. Paris: Hermann et Cie.

—— 1951. Introduction to biochemistry of protozoa. In *Biochemistry and Physiology of Protozoa*, Vol. 1, pp. 1–26. Ed. A. Lwoff. New York: Academic Press Inc.

McLaughlin, J. J. A. & Provasoli, L., 1957. Nutritional requirements and toxicity of two marine *Amphidinium*. *J. Protozool.*, Vol. 4, Suppl. p. 7.

Pascher, A., 1914. Ueber Flagellaten und Algen. *Ber. dtsch. bot. Ges.*, Bd. 32, pp. 136–60, 430.

Pringsheim, E. G., 1935. Über Azetatflagellaten. *Naturwissenschaften*, Bd. 23, pp. 110–14.

—— 1952. On the nutrition of *Ochromonas*. *Quart. J. micr. Sci.*, Vol. 93, pp. 71–96.

Pringsheim, E. G. & Hovasse, R., 1948. The loss of chromatophores in *Euglena gracilis*. *New Phytol.*, Vol. 47, pp. 52–87.

Provasoli, L., 1938. Studi sulla nutrizione dei protozoi. *Boll. Zool. agr. Bachic.*, Vol. 9, pp. 1–124.

—— 1957. Alcune considerazioni sui caratteri morfologici e fisiologici delle alghe. *Boll. Zool. agr. Bachic.*, Vol. 22, pp. 143–88.

—— 1958. Nutrition and ecology of protozoa and algae. *Annu. Rev. Microbiol.*, Vol. 12, pp. 279–308.

Provasoli, L., McLaughlin, J. J. A. & Droop, M. R. 1957. The development of artificial media for marine algae. *Arch. Mikrobiol.*, Bd. 25, pp. 392–428.

Reinert, J. & White, P. R., 1956. The cultivation *in vitro* of tumor tissues and normal tissues of *Picea glauca*. *Physiol. Plant.*, Vol. 9, pp. 177–89.

Rose, W. C., 1938. The nutritive significance of the amino-acids. *Physiol. Rev.*, Vol. 18, pp. 109–36.

Snell, E. E., 1951. Bacterial nutrition—Chemical factors. In *Bacterial Physiology*, pp. 215–56. Ed. C. W. Werkman and P. W. Wilson. New York: Academic Press Inc.

Spencer, C. P., 1958. The chemistry of ethylenediamine tetra-acetic acid in sea water. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 127–44.

Stephenson, M., 1949. *Bacterial Metabolism*. 398 pp. 3rd edition. London: Longmans, Green and Co.

Storm, J. & Hutner, S. H., 1953. Nutrition of *Peranema*. *Ann. N.Y. Acad. Sci.*, Vol. 56, pp. 901–9.

Trager, W., 1953. Nutrition. In *Insect Physiology*, pp. 350–86. Ed. K. D. Roeden. London: Chapman and Hall.

White, P. R., 1951. Nutritional requirements of isolated plant tissues and organs. *Annu. Rev. Pl. Physiol.*, Vol. 2, pp. 231–44.

J. mar. biol. Ass. U.K. (1959) 38, 621–627
Printed in Great Britain

THE BIOLOGY OF THE PRAWN, *PALAEMON* (=*LEANDER*) *SERRATUS* (PENNANT)

By G. R. Forster

The Plymouth Laboratory

In a recent paper Cole (1958) has offered a new interpretation of the results obtained in 1949 and 1950 during my investigations of the biology of prawns. The present paper is the outcome of a re-examination of these results and of further prawn measurements which were continued on a small scale until 1955. The later investigations consisted of an annual survey of the prawns, particularly the O-group, caught from the rocks on the south side of the R.A.F. Station, Mount Batten, usually during the first low spring tides every October. The total number of prawns measured in the whole period was over 8,600.

THE 1949 AND 1950 SAMPLES

The length measurements of the prawns caught during a 12-month period, starting in October 1949, have been re-analysed into millimetre instead of ½-centimetre groups and the results plotted on probability paper. As Harding (1949) has pointed out, this paper can help considerably in the analysis of polymodal samples; though with the prawn's long breeding season one cannot necessarily expect year-groups to be sharply defined. During the winter, catches have been combined and plotted at monthly or bimonthly intervals, but during the summer when rapid growth may occur it was found desirable to limit the inclusive period for any one group to a maximum of about a fortnight.

The results are shown in Fig. 1, with the different symbols indicating my interpretation of the year-groups. The appearance of the O-group prawns in August and September can be clearly distinguished both with males and females. In July and August there were also many tiny prawns taken by a stramin net which could not be sexed. These have not been included.

The September samples of the O-group appear in Fig. 1 as reasonably straight lines, i.e. showing a normal distribution. But by October, after further growth and recruitment, the population has been widely spread and when plotted can be considered as two or three separate but overlapping populations. These populations probably represent fluctuations in the numbers of larvae settling in the early part of the summer. A separate late 'brood' in the smallest stages is clearly shown with the males in October 1949, and with the females in October 1950. Cole (1958, p. 20) preferred to consider these 3-cm prawns as the true O-group; but they only represent about

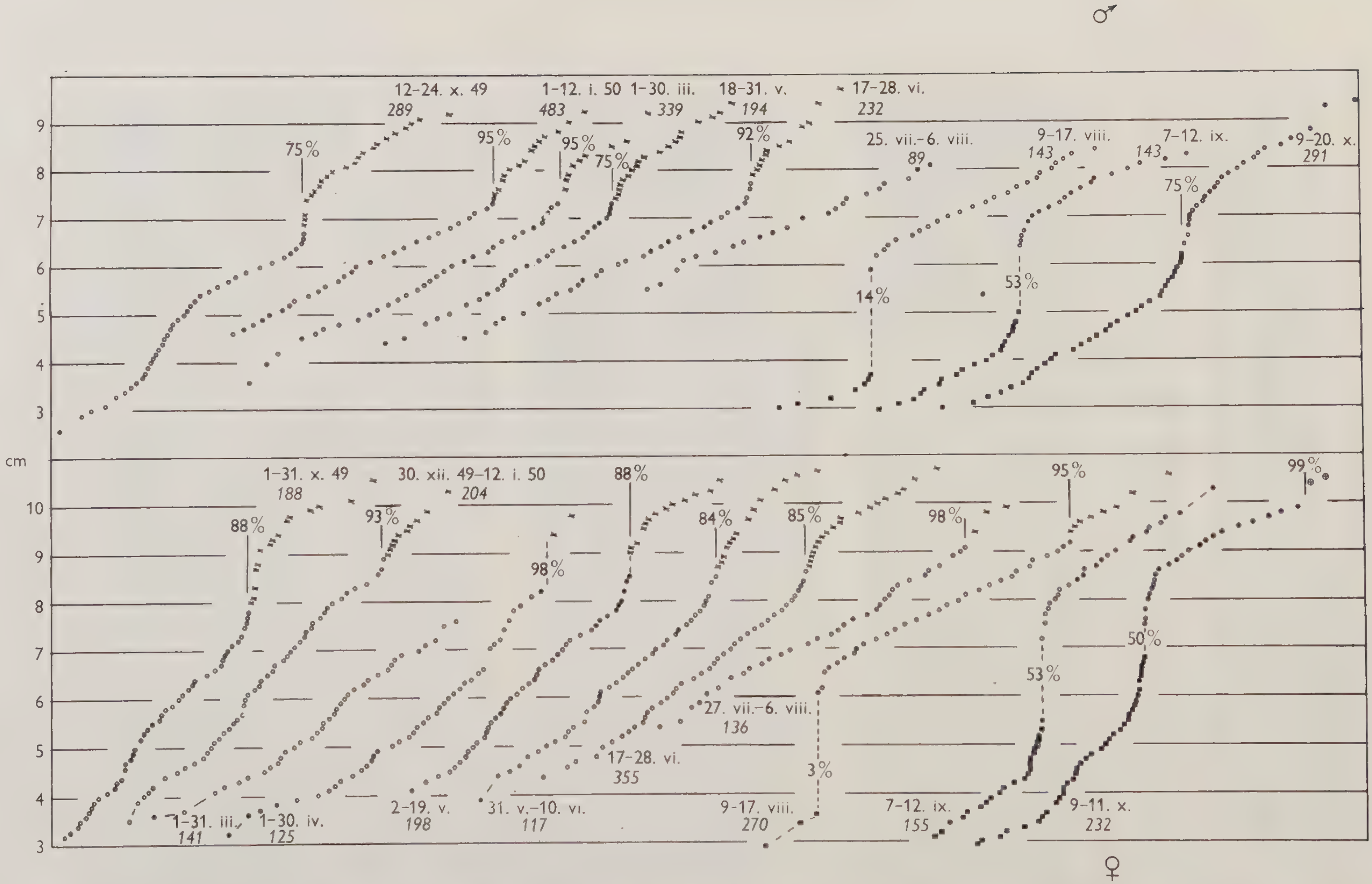


Fig. 1. Catches of male and female prawns in 1949 and 1950 plotted as cumulative percentages on log scale (probability paper). The year-groups from 1948–50 are represented by crosses, open circles and solid dots respectively.

5% of the total catch, and are even smaller than the clearly defined O-group of later, colder years. The 1949 males soon grew sufficiently to merge with the main bulk of the O-group. The exceptionally warm conditions during the spring and summer of 1949 probably led to a very early settlement of larvae, beginning in May or June. Lebour (1947), during her 1940–5 investigations, found larvae all the year round except in November and December, but with the greatest numbers in June, July and August.

The sequence of replacement of the 1948 and 1949 year-groups can be seen in Fig. 1 and calls for no detailed re-description. It is possible that some of the prawns included in the 1948 year-group might in fact be somewhat older; in particular the January 1950 catch of males shows two populations at 8 cm and 9 cm but these numbers are very small; the distinction is not evident for several months and the whole group disappear from the catches after the end of June. The 1948 group females continued to appear in the August samples, i.e. after their third birthday. But they too had apparently died off by mid-September, though two at 10·4 cm and 10·5 cm caught in October might represent the last survivors; however, they form only 1% of the total sample.

CATCHES FROM 1951–1955

In 1952 and 1955 only the O-group were sampled, but for the other three years large as well as small size-groups were measured. The results are shown in histogram form in Fig. 2. Both males and females exhibit very clear bimodal distributions, the O-group appearing at 3–4 cm and the 1-year-old at 7–8 cm. The 1-year-old females have outgrown the males by about 1 cm. In 1951 there is a small 'tail' of very large prawns. When their distribution is plotted on probability paper, these prawns show up clearly as a separate group and are presumably the few surviving 2-year-olds. Their numbers have dwindled to less than 2% of the total. The various O-group distributed in Fig. 2 are all much smaller than their counterparts in 1949–50 but correspond closely to the results of Cole from N. Wales in the same period. The mean lengths of the O-group for each year 1949 to 1955 have been extracted and plotted together with the sea temperature in Table 1.

The figure for 1952 is approximate as only the percentages of each ½-cm group were available, the original measurements having been lost. The temperatures have been taken from Cooper (1958). From the Table it can be seen that there is a general agreement between the temperatures and lengths particularly in the two warmer years 1949 and 1950. Mr. G M. Spooner had kindly undertaken a statistical analysis of the figures and has found a significant correlation between them.

Fig. 2 also shows a sample of prawns taken in July 1954. In both sexes there are two distinct year-groups which are assumed to be those of 1953 and 1952, with the 1953-group females having grown considerably since the

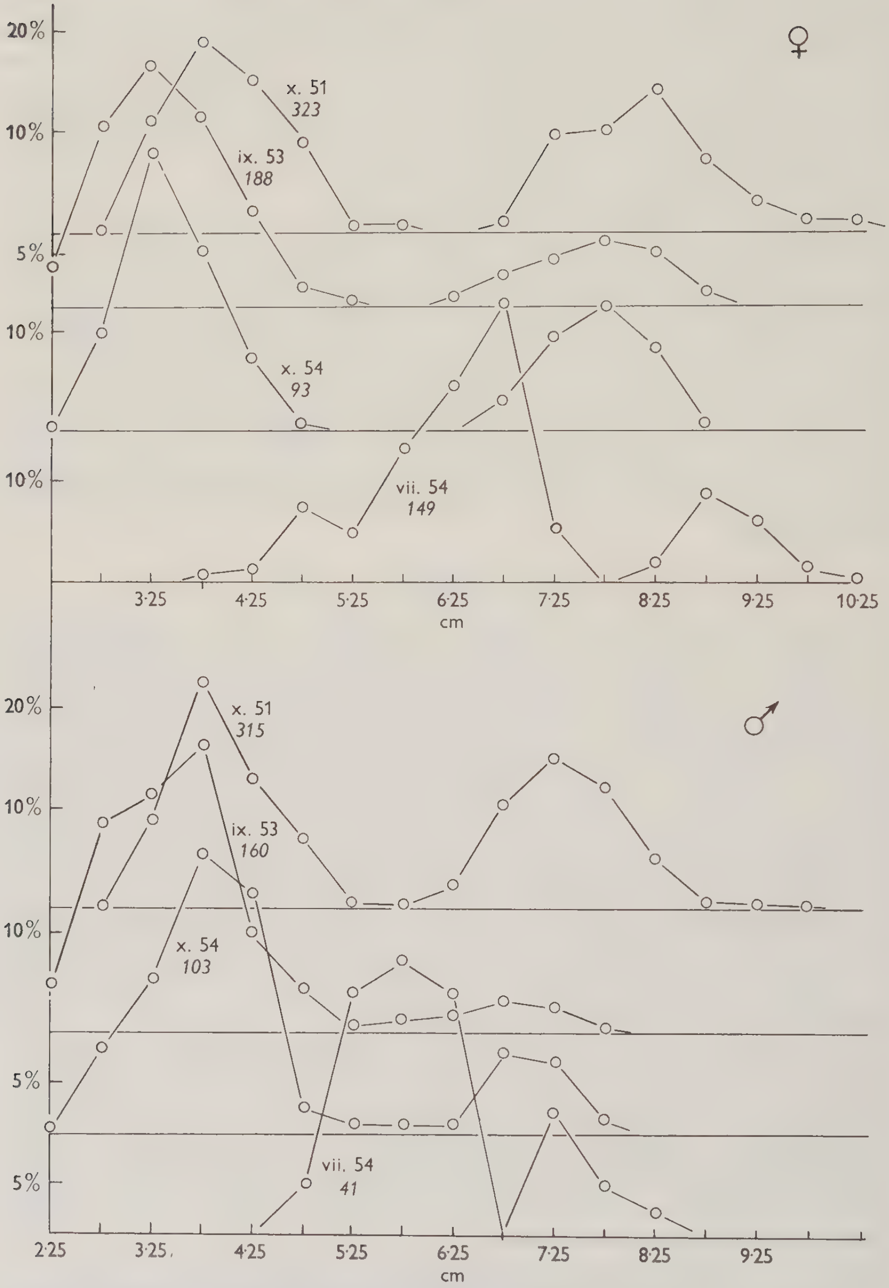


Fig. 2. Samples of male amd female prawns from Rum Bay, 1951–4, in ½-cm groups.

previous autumn. Table 2 gives the breeding condition of these female prawns.

It is very probable that some of the non-breeding prawns about 6 cm in length had already hatched one brood, and also the two-year-olds (7–8 cm) would be carrying their second brood.

TABLE 1

Year	Mean length of O-group. Oct. sample ♂+♀	No. in sample	Difference ±39 mm (mm)	Agg. temp.* (°C)
1949	56·1	366	+17·1	+9·6
1950	47·5	332	+8·5	+3·6
1951	38·6	354	−0·4	−2·8
1952	39·9		+0·9	+1·4
1953	35·9	104	−3·1	−0·6
1954	35·5	148	−3·5	−3·2
1955	37·9	197	−1·1	−1·8

* Temperature heading: aggregate differences of each monthly mean sea temp. for March–October from 10-year average 1947–56.

TABLE 2. PRAWNS CAUGHT ON 2 JULY 1954

Length (cm)	Non-breeding	Total berried	Berried with eggs near hatching	Cemented
3·5–3·9	1	0	0	0
4·0–4·5	2	0	0	0
4·5–4·9	11	0	0	0
5·0–5·4	7	0	0	0
5·5–5·9	8	11	3	1
6·0–6·4	12	16	9	1
6·5–6·9	23	17	11	2
7·0–7·4	4	3	3	1
7·5–7·9	0	0	0	0
8·0–8·4	0	3	0	0
8·5–8·9	4	9	1	0
9·0–9·4	0	8	1	1
9·5–9·9	0	2	1	0
10·0–10·4	0	1	1	1

The 1953 year-group will have spawned in April or May and must therefore have continued to grow rapidly in the late autumn. Its mean length in July was 61 mm. The small non-breeding prawns would have been growing in May and June as would the larger ones after hatching their eggs, so the mean length of the whole group may be estimated at about 55 mm before spawning. The group therefore grew from a mean length of 32 mm at the end of September to 55 mm in the spring. At first sight this might appear an impossibly high rate of growth for the colder part of the year. However, in the fortnight from September 1953, when the sample was taken, until 9 October, the mean length of the group increased by 4 mm; as the sea temperature did not decline rapidly until January, a length of 50–55 mm might well have been attained by the end of December.

DISCUSSION

Apart from the particularly warm years 1949 and 1950 the growth rate of prawns at Plymouth is comparable to that found by Cole (1958) in N. Wales. The O-group in October averages about 38 mm total length and 7 mm carapace length. There is a marked difference in the age at which the female prawns mature. Whereas in Wales only a few females breed in their first year, at Plymouth the bulk of the female population spawn before their first birthday.

The breeding period seems, in general, to be a little earlier than that found by Cole (1958) at Holyhead in 1953, but much later than at Plymouth in the warmer year, 1950. By their second October there is little difference in size between the Plymouth prawns and their Welsh counterparts.

Cole (1958) questions my earlier interpretation of the autumn 1949 and 1950 catches, maintaining that it involved an impossibly high growth rate because of a large size difference between samples at the end of August and the early part of September. But the August samples were taken with a stramin net which would not catch nearly as many of the 3–4 cm prawns as the normal hand net with $\frac{1}{2}$ in.-mesh netting. The O-group samples taken in August, September and October shown in Fig. 1 present a consistent pattern of moderately rapid growth, together with an increasing spread as later settling juveniles come into the samples. Nor is there any trace of the group suggested by Cole to lie between my O-group–1-year-old group, either in 1949–50 (Fig. 1) or in the later years, Fig. 2.

With regard to the older prawns which Cole considers to live several years longer than at Plymouth, it is difficult to follow the interpretation of his results. Concerning the males (p. 12) he states 'a group of prawns completing their second year with a peak at 13·0–14·2 mm in June can also be discerned,' but in the next paragraph Cole describes 'the group at 14·8 mm in June as attaining 3 years in July.' In my view these groups are so similar in size that they should both be considered as nearly two years old. Similarly with the female prawns it seems that 'the strong group about 16·6 mm in June' described on p. 15 could more reasonably be regarded as nearly two years old rather than three. Cole describes his fig. 13 as providing clear evidence of the existence of at least 5 year-groups, even though the older groups are merged, but by comparison with his fig. 12 the main bulk of catch in fig. 13 (October–November) would seem to be composed of 1 and 2-year-olds and the few larger prawns both in this and other samples of female prawns would, to my mind, be more reasonably taken as the remnants of the 3-year-old group rather than 4 or 5-year-olds. The largest prawns of all might well be somewhat older but they form a very small proportion of the catches.

SUMMARY

Data on the age and growth of the prawn *Palaemon serratus* (Pennant) collected in 1949 and 1950 have been re-examined in the light of further investigation at Plymouth from 1951–5 and of Cole's results from North Wales. Contrary to Cole's view the original interpretation of the growth rate is considered to be valid.

The length of the O-group in October is significantly correlated with the sea temperatures from March till October. Growth rates of the Plymouth and North Wales population are similar but the females mature much sooner at Plymouth. The age reached by the N. Wales prawns is thought to be rather less than Cole has suggested.

REFERENCES

Cole, H. A., 1958. Notes on the biology of the common prawn. *Fish. Invest., London*, Ser. 2, Vol. 22, No. 5

Cooper, L. H. N., 1958. Sea temperatures in Plymouth Sound. *J. mar. biol. Ass. U.K.*, Vol. 37, pp. 1–3. 22 pp.

Forster, G. R., 1951. The biology of the common prawn, *Leander serratus* Pennant. *J. mar. biol. Ass. U.K.*, Vol. 30, pp. 333–60.

Harding, J. P., 1949. The use of probability paper for the graphical analysis of polynodal frequency distributions. *J. mar. biol. Ass. U.K.*, Vol. 28, pp. 141–53.

Lebour, M. V., 1947. Notes on the inshore plankton of Plymouth. *J. mar. biol. Ass. U.K.*, Vol. 26, pp. 527–47.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

DENTON, E. J., 1959. The contributions of the orientated photosensitive and other molecules to the absorption of whole retina. *Proc. roy. Soc.*, B, Vol. 150, pp. 78–94.

Various animals were chosen to give golden, red and purple retinae. It is confirmed, by a method giving the absorption curve of unbleached retina, that deep-sea fish have golden photosensitive pigments in densities so high that they absorb over 90% of blue-green light striking the retina. Although light has passed through all retinal layers the spectral total density curves correspond closely to those obtained on highly purified retinal extracts. After making a small correction for losses of light in the layers of retina other than the receptor layer the $D_{\text{min.}}/D_{\text{max.}}$ ratios were about 0·25 for chrysopsin retinae and 0·45 for porphyropsin retinae. The losses of light in retinae from which the rod and cone layer has been brushed off, although varying little with wavelength, rise slowly in the near ultra-violet and red from a minimum in the yellow.

The edge-fold preparation, useful for studying the dichroism of the retinal rods is described. It is shown that the spectral curves of dichroic difference in density are very close to those of total retinal density and therefore there can be little material absorbing light between 400 and 720 mμ orientated along the axes of unbleached rods. There is such material absorbing near ultra-violet light present in unbleached retinae whose principal photosensitive pigment is derived from vitamin A_2. For both vitamin A_1 and vitamin A_2 retinae the sense of the dichroism in the near ultra-violet is reversed in the first hour following the bleaching of the photosensitive pigments. This shows that the molecules of vitamin A in the bleached isolated retina are orientated with their axes of resonance parallel to the axes of the rods, a conclusion confirmed by studies of the polarization of the fluorescence of visual white in the bleached retina.

E.J.D.

MURRAY, R. W., 1959. The response of the ampullae of Lorenzini to combined stimulation by temperature change and weak direct currents. *J. Physiol.*, Vol. 145, pp. 1–13.

When single units in the ampullae of Lorenzini are stimulated by weak direct currents their impulse frequency varies linearly with current in most preparations. When thermal stimuli are applied during the adapted response to maintained D.C. the change in frequency due to the combined stimuli equals the sum of the changes due to each separately. When the unadapted response to D.C. is combined with the thermal response, interaction occurs. The direction of the spatial gradient of temperature is not significant for the thermal response. Some anomalous thermal responses are described.

R.W.M.

NICHOLS, D., 1959. Changes in the Chalk heart-urchin *Micraster* interpreted in relation to living forms. *Phil. Trans.*, B, Vol. 242, pp. 347–437.

Seven species of irregular echinoids, whose morphology, behaviour and ecology show important comparative features, were studied, and many aspects of their mode of life, in particular their burrowing, feeding, sanitation and locomotion, are shown to be closely correlated with the particle-sizes of the substrata in which they live, and this adaptation is expressed in many features of their tests, in particular the position and degree of development of the fascioles and the division of labour in the tube-feet.

The fossil heart-urchin *Micraster* is one of the best examples of continuous and directional evolution of individual characters, and the study of living forms has been used to suggest the changes in mode of life which accompanied the structural changes. The main change appears to have been a gradual increase in the depth to which the Micrasters burrowed below the sea-floor. When the shallow-burrowing forms ceased to occur, another closely-related form, *Isomicraster*, arose in the English area, probably by immigration. By analogy with living forms, in particular the number and arrangement of tube-feet in the petaloid parts of the paired ambulacra, this form most likely lived only partially submerged in the substratum. The taxonomy of the English *Micraster* complex is discussed in the light of this approach. D.N.

SPOONER, G. M., 1959. New members of the British marine bottom fauna. *Nature, Lond.*, Vol. 183, pp. 1695–6.

Some unfamiliar small invertebrates are recorded from bottom gravels off Plymouth, more particularly the Eddystone shell gravel (at depth of about 25 fm.). Acochlidiacean gastropods are reported from Britain for the first time and represented by *Microhedyle lactea* Hertling, *Hedylopsis suecica* Odhner, and *Philinoglossa helgolandica* Hertling. The holothurian *Leptosynapta minuta* (Becher) was also found.

The Eddystone shell gravel harbours one oligochaete and five species of mites, including *Scaptognathas tridens* Trouess. and *Halacarus bisulcus* Viets which are new to Britain; and, furthermore, it is particularly rich in small malacostracans. Out of 56 species listed, 20 are new to the Plymouth Fauna and five or six new to science.

Tanaidacea are dominated by *Typhlotanais microcheles* Sars and *Strongyurella indivisa* Hansen. Isopoda include *Paramunna bilobata* Sars, *Eurycope pygmaea* Sars, *Microjaera anisopoda* Bocquet and Lévi, and *Microcharon harrisi* Spooner. The last belongs to a genus of small blind colourless forms of which six of the nine species recognized live in terrestrial ground-water in southern and eastern Europe, and are truly 'interstitial' or 'phreatic'.

Among many Amphipoda, there is a new metopid and a new syrrhoid, but the most interesting and unexpected finds were representative of two cosmopolitan 'phreatic' families—Bogidiellidae and Ingolfiellidae. The bogidiellid will require a new generic name. Previously species have been found in fresh water or coastal sand of southern Europe or Brazil. The genus *Ingolfiella* includes six species from the most diverse range of habitats (from the abyssal Atlantic to a cave in the Belgian Congo): the two specimens from the Eddystone are very close to *I. acherontis* Karaman from the Macedonian uplands.

G.M.S.

BOOK REVIEW

ENDOCRINE CONTROL IN CRUSTACEANS

BY D. B. CARLISLE AND SIR FRANCIS KNOWLES

Cambridge University Press, 1959.

This is Vol. 10 of the *Cambridge Monographs in Experimental Biology* and it is a worthy member of a series of publications of great merit.

Thirty years ago Perkins and Koller discovered the endocrine mechanism of colour change in the Crustacea, and when a few years later Hanström demonstrated that the sinus gland was an endocrine organ, the scene was set for a new epoch in comparative endocrinology.

The authors sub-divide progress in this field into three phases. During the first phase the nature of the newly discovered chromactivating principles associated with the sinus gland was further substantiated. The second phase brought to light the fact that functions other than chromatic mechanisms are under hormonal control, and the third phase, seemingly approaching its end now, resulted in our knowledge that modified neurons act as producers of hormones, and that hormone transport takes place along nerve axons.

Of the achievements during these three periods of research the authors give a lucid and excellently illustrated account, the authoritative nature of which stems from the fact that both have made substantial original contributions to our knowledge in this field.

There are chapters on the neurosecretory system of the head and thorax, on the mechanism of colour change, on the pericardial organs and their influence on the heart beat, on the hormonal control of growth, moulting, development, metabolism, and breeding. In conclusion the authors point to the future fourth phase of progress and look forward to the extension of research beyond the decapod crustaceans and to a synthesis of the results with those gained in other fields of endocrinology.

There is an extensive list of references and a very useful index. O.E.L.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1958–59

The Council have to report with deep regret the death of Dr G. A. Steven, F.R.S.E., who had been a member of the Scientific Staff of the Plymouth Laboratory since 1928. During this time he did much to develop the sea-going work of the laboratory, and made notable contributions to the biology of fishes.

The Council also regret to report the deaths of Mrs E. W. Sexton, who had been a member of the staff of the Plymouth laboratory for many years, and was especially well known for her research on amphipod crustacea; of Dr Å. Vedel Tåning, President of the International Council for the Exploration of the Sea, who was an Honorary Member of the Association; and of Prof. James Ritchie, C.B.E., LL.D., who had been a member of Council.

THE COUNCIL AND OFFICERS

Four ordinary meetings of the Council were held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was nineteen.

THE PLYMOUTH LABORATORY

During the year a small building was erected in the laboratory grounds for the storage of inflammable chemicals. The passages of the north building and the bridge connecting the two buildings have been redecorated; this has also necessitated considerable electrical rewiring.

AQUARIUM

The aquarium attracted unusual numbers of the public during the summer, probably because of the wet weather, the attendance on August Bank Holiday being a record.

The tank room was closed to the public on 27 September 1958, and work on the modernization and reconstruction of the tanks is well in hand.

RESEARCH SHIPS

The research ships 'Sarsia' and 'Sula' have operated regularly throughout the year apart from normal overhauls. In November and December 1958 M.L. 'Gammarus' was completely overhauled. Her woodwork was generally in very good condition. A fibre-glass dinghy and an outboard motor have been purchased for use with the 'Gammarus'.

R.V. 'Sarsia', Lt. Cdr. C. A. Hoodless, D.S.C., R.N.R. in command, has now been in commission for five years, and has made many cruises over an area stretching from the eastern end of the English Channel to the continental slope south-west of Ireland and off the coast of Spain. The interesting results of the extension of the Plymouth laboratory's working area, and of the facilities for doing physiological research at sea are now becoming apparent.

R.V. 'Sula', Mr W. J. Creese in command, has been in commission for over ten years. With the increasing number of cruises undertaken annually by R.V. 'Sarsia' the major part of the daily collecting of specimens is now done by R.V. 'Sula'.

The collection of specimens from inshore waters and intertidal areas continues to be undertaken by M.L. 'Gammarus' under the charge of Mr A. C. Briggs.

STAFF

On 1 October 1958 Mr W. H. Searle, B.E.M., retired from the staff of the Plymouth laboratory, after 63 years of devoted service to the Association. He will have the best wishes of all members of the Association and of the many visiting research workers for whom he has collected specimens. The building up of our knowledge of the fauna from its early beginnings owes much to his skill and powers of observation. He will be remembered with affection by all.

Dr L. H. N. Cooper and Mr G. M. Spooner have been promoted to the grade of Senior Principal Scientific Officer as from 1 April 1958.

Dr D. B. Carlisle has been promoted to the grade of Principal Scientific Officer as from 1 April 1958.

Dr T. I. Shaw joined the staff of the Plymouth laboratory as Senior Scientific Officer on 1 September 1958.

Mr G. W. Bryan joined the staff of the Plymouth laboratory on 1 October 1958 in a special temporary appointment.

Mr Q. Bone joined the staff of the Plymouth laboratory as Scientific Officer on 1 January 1959.

Dr B. C. Abbott left the staff of the Plymouth laboratory on 1 September 1958 to continue his appointment in the University of California at Los Angeles.

Dr Mary Parke attended the Third International Seaweed Symposium held in Galway, Eire, in August 1958.

Dr E. J. Denton attended the meeting of the International Council for the Exploration of the Sea held in Copenhagen in September and October 1958.

Dr D. B. Carlisle spent a month in August and September 1958 working at the Kristineberg Marine Laboratory, Sweden.

Dr A. J. Southward spent seven weeks in August and September 1958 making a survey of the distribution of barnacles round the Irish coasts.

Through the kindness of the Director of the National Institute of Oceanography Mr P. G. Corbin and Dr L. H. N. Cooper took part in cruises of R.R.S. 'Discovery II' in April and November to December 1958 respectively. In March 1959, through the kindness of the Director of Fisheries Research, Ministry of Agriculture, Fisheries and Food, Dr A. J. Southward accompanied a cruise of the fisheries research vessel 'Sir Lancelot'.

International Paints Research Fellowship

The Directors of International Paints Ltd. have, by arrangement with the Council of the Association, continued the endowment of their Research Fellowship at the Plymouth laboratory for a further three years.

Dr G. T. Boalch has been appointed to the Fellowship, and he started work at Plymouth on 1 October 1958.

Occupation of Tables

The following one hundred and sixty workers have occupied tables at the Plymouth laboratory during the year:

E. Adams, Plymouth (Library).
R. McN. Alexander, Cambridge (Swim bladders of fish).
Dr J. S. Alexandrowicz, Plymouth (Invertebrate nervous systems).
F. P. Anderson, Cape Town (Physical oceanography).
S. R. Armstrong, Bryanston School, Dorset (Mollusca).
Dr Daphne Atkins, Plymouth (Ciliary mechanisms of brachiopods).
Miss D. Ballantine (Mrs B. T. Hepper), Conway (Dinoflagellates).
W. J. Ballantine, London (*Patella*).
Dr B. McK. Bary, Edinburgh (High-speed plankton sampling).
Dr Elizabeth J. Batham, Cambridge (Nervous system of *Metridium*).
Dr J. H. Belcher, London (Bangioideae and Nemalionales).
E. Bernabe, Philippines (Plankton).
Miss M. Beynon, Aberystwyth (General).
Dr Anna M. Bidder, Cambridge (Biology of cephalopods).
Dr G. T. Boalch, International Paints Research Fellow (Effects of toxic substances on *Ectocarpus*).
Dr B. P. Boden, La Jolla (Penetration of light and vertical migration of plankton).
Q. Bone, Oxford (Feeding behaviour of *Amphioxus*).
A. D. Boney, Plymouth (Ecology of red algae).
Dr J. S. Bradshaw, La Jolla (Ecology of estuarine Foraminifera).
L. R. Brightwell, Truro (Holothurians)
R. W. Brimblecombe, Porton (Shark repellants).
J. D. Bromhall, Hong Kong (Underwater photography).
Dr Eleanor M. Brown, London (Plankton).
Dr P. C. J. Brunet, Oxford (*Chaetopterus* and Pogonophora tubes).
Dr A. J. Bruce, Glasgow (Penaeid prawns).
B. M. H. Bush, Cambridge (Crustacean neuro-muscular physiology).
Mrs P. A. Caldwell, Plymouth (Library).

Dr P. C. Caldwell, Alan Johnston, Lawrence and Mosely Research Fellow of the Royal Society (Muscle and nerve physiology).
C. Carré, Villefranche-sur-Mer (Culture of phytoplankton).
Prof. M. R. Carriker, North Carolina (Malacology).
Prof. L. S. Ciereszko, Oklahoma (Vanadium in ascidian eggs and larvae).
Miss E. Clay, Brixham (Library).
Dr H. A. Cole, Lowestoft (Saltash oyster fishery).
J. W. Coles, London (Seaweed nematodes).
J. S. Colman, Port Erin (Vertical distribution of oceanic plankton).
Dr J. D. Costlow, North Carolina (Barnacles).
C. A. Cosway, Torquay (Library).
Dr Wilhelmina A. M. Courtney, Nottingham (*Amphioxus*).
C. J. Croft, Plymouth (Library).
Dr R. I. T. Cromartie, Cambridge (Pigments of *Antedon*).
J. G. C. Cubb, Winfrith Heath (Analysis of sea-water).
K. W. Daisley, Shinfield (Vitamin B_{12} in sea-water).
Dr R. Phillips Dales, London (Coelomic cells of *Sabella*).
J. B. Davies, Oxford (General).
E. W. Dawson, Cambridge (Hydrostatic skeleton in invertebrates).
P. S. B. Digby, London (Zooplankton).
Miss E. J. Dimelow, New Brunswick (Biology of *Antedon*).
J. D. Dodge, London (Cytology of Protista).
Dr D. T. Donovan, Bristol (Submarine geology).
Dr Patricia L. Dudley, Seattle (Notodelphyid copepods).
Dr R. Endean, Bristol (Role of vanadocytes in tunicin formation).
J. Ernst, Paris (Underwater study of algae).
Dr L. Euzet, Sète (Platyhelminths of fish).
Dr Maria M. Felinska, Nottingham (Ciliates).
Dr Una Fielding, London (Central nervous system of fish).
Prof. P. H. Fischer, Paris (*Otina*).
Dr L. R. Fisher, Shinfield (Pigments of invertebrate eyes).
D. N. Fletcher, Harwell (Trace analysis).
Prof. E. Florey, Seattle (Crustacean inhibitory nerve fibres).
I. H. Ford, Bristol (Submarine geology).
P. Foxton, National Institute of Oceanography (High-speed plankton sampling).
R. F. H. Freeman, London (Metabolism of *Scrobicularia*).
Dr Vera M. Fretter, Reading (Prosobranchs).
Dr M. M. Frodyma, Hawaii (Chemistry of sea-water).
M. H. W. Gall, London (Marine photometry).
Dr J. B. Gilpin-Brown, Plymouth (Biology of nereids; pelagic squid).
Miss E. H. Goldie, Shinfield (Pigments of invertebrate eyes).
Prof. A. Graham, Reading (Prosobranchs).
Dr E. C. Haderlie, Monterey and Bristol (Ecology of polychaetes).
D. N. F. Hall, Colonial Office (Indo-West-Pacific Penaeidae).
Prof. Sir Alister C. Hardy, F.R.S., Oxford (Drawing marine animals).
M. G. Hardy, Reading (Nerve histology of lamellibranch siphons).
Prof. J. E. Harris, F.R.S., Ray Lankester Investigator, Bristol (Vertical migration of plankton).
Dr H. W. Harvey, F.R.S., Plymouth (Productivity of sea-water).
D. Heddle, Oxford (Asteroid skeletons).
Miss M. Henderson, London (Shore ecology).
B. T. Hepper, Conway (Lobster marking).

Prof. A. L. Hodgkin, F.R.S., Cambridge (Nerve physiology).
M. Hornsey, Cambridge (Light reactions in *Procerodes*).
Dr G. M. Hughes, Cambridge (Respiratory movements of teleosts).
O. D. Hunt, Newton Ferrers (Library).
Dr G. M. Jarman, Bristol (Vertical migration of plankton).
Dr C. H. Jellard, Plymouth (Library).
Dr Penelope M. Jenkin, Bristol (Library).
Dr J. B. Jennings, Leeds (Feeding and digestion in Nemertea).
Dr R. J. Jones, Croydon (Drawing fish).
Dr Joanna M. Kain, Port Erin (Sublittoral algae).
Dr Elizabeth M. Kampa (Mrs B. P. Boden), La Jolla (Penetration of light and vertical migration of plankton).
Dr P. Karlson, Munich (Chromactivating hormones).
Dr G. Y. Kennedy, Sheffield (Sponge regeneration; chlorophyll pigments).
Prof. H. Kinosita, Tokyo (Ciliary response in invertebrates).
Dr P. L. Kramp, Copenhagen (Medusae).
Dr S. Krishnaswamy, Southampton (Respiratory metabolism of parasitic copepods).
Dr K. Lagerspetz, Turku, Finland (*Mytilus edulis*).
Miss E. C. Leach, Cambridge (Discharge of nematocysts of *Actinia*).
Dr Marie V. Lebour, Plymouth (Decapod larvae).
Prof. B. Levring, Göteborg (Algae).
Dr J. Llewellyn, Birmingham (Trematode parasites of fishes).
Prof. O. E. Lowenstein, F.R.S., Birmingham (Elasmobranch labyrinths).
Miss M. R. Lunt, Oxford (Biochemistry in arthropods).
Prof. Irene Manton, Leeds (Flagellates and motile cells of algae).
A. L. Martin, London (Digestive gland in *Gammarus*).
Dr A. B. Meggy, Plymouth (Library).
Dr N. A. Meinkoth, Swarthmore, Pennsylvania (Cestodes).
Prof. J. L. Mohr, Los Angeles (Protozoan parasites of Crustacea; Mesozoa).
Dr J. E. Morton, London (Pleurobranchoids).
Dr R. W. Murray, Birmingham (Sensory physiology of rays).
Dr E. Naylor, Swansea (Isopods).
Dr Margaret Naylor, Hull (Cytology of Phaeophyceae).
Dr F. Öztig, Banyuls (Red algae).
Dr C. F. A. Pantin, F.R.S., Cambridge (Fine structure of *Metridium*).
Dr A. M. Patil, Reading (Taxonomy of Rissoidae).
Mrs J. L. Pearson, Cambridge (Embryology of *Sycon*).
C. J. Pennycuick, Cambridge (Physiology of dogfish muscle).
W. J. Phillipps, Wellington, N.Z. (Teeth of spiny dogfish).
Dr W. T. W. Potts, Birmingham (Free amino acids in *Gammarus*).
Cdr. C. F. B. Powell, R.N. (Rtd.), Plymouth (Library).
J. D. Pye, London (Colour change in fishes).
A. M. Qureshy, London (Haemogregarines of fishes).
Miss P. M. Ralph, Wellington, N.Z. (Hydroids and medusae).
Miss M. Razarihelisoa, Madagascar (Trematode parasites of fish).
Dr W. J. Rees, London (Cnidaria of sand and mud).
Dr J. D. Robertson, Glasgow (Chemistry of cephalopod muscle)
Prof. M. Rockstein, New York (Echinoderm orientation and photosensitive pigments).
G. Rodriguez, Caracas, Venezuela (Ecology of sandy shores).
Dr D. M. Ross, London (Behaviour of *Calliactis*).
Prof. Findlay E. Russell, Los Angeles (Toxins of weevers).

Prof. P. SAWAYA, São Paulo (Body fluid of Holothuria).
D. J. SCARRATT, D.S.I.R. (Fauna of *Laminaria* holdfasts).
Prof. K. SEMBRAT, Breslau (Dogfish thyroid).
Prof. Z. SEMBRATOWA, Breslau (Neurosecretion in Nemertini and Polychaeta).
A. B. SIDLE, London (Algae).
A. C. SIMPSON, Lowestoft (Saltash oyster fishery).
R. J. SKAER, Cambridge (Rhabdite discharge in *Procerodes*).
Dr DOROTHY M. SKINNER, Harvard (Crustacean moult cycle).
Dr EVE C. SOUTHWARD, Plymouth (Pogonophora; polychaetes).
B. W. SPARROW, Newton Ferrers (Library).
Miss F. A. STANBURY, Plymouth (*Cladophora*).
Dr D. C. STEWART, Chicago and Harwell (Trace analysis).
Dr J. H. STOCK, Amsterdam (Crustacean parasites of invertebrates).
Miss E. M. F. SWALE (Mrs J. H. BELCHER), London (Bangioideae and Nemalionales).
R. V. TAIT, London (Shore ecology and plankton).
Prof. E. TORTONESE, Genoa (Channel fauna).
M. TUFAIL, Exeter (Development of *Porcellana*).
Dr J. VERWEY, den Helder (Library).
Dr H. G. VEVERS, London (Library).
G. E. WALSTER, Plymouth (Glycolysis in *Maia*).
Surg.-Cdr. G. WEDD, R.N., Porton (Shark repellants).
Prof. G. P. WELLS, F.R.S., London (*Arenicola*).
Dr J. O. WERSÄLL, Stockholm (Elasmobranch labyrinth).
Dr H. P. WHITING, Bristol (Nervous system of *Amphioxus* and *Scyliorhinus*).
Prof. W. F. WHITTARD, F.R.S., Bristol (Submarine geology).
J. H. WICKSTEAD, Colonial Office (Tropical plankton).
Prof. C. A. G. WIERSMA, Pasadena (Proprioreceptors in crustacean legs).
Dr H. HARFORD WILLIAMS, Aberystwyth (Cestodes).
Dr D. I. WILLIAMSON, Port Erin (Decapod larvae).
Dr SHEILA M. WILLMOTT, London (Digenea of fish).
Dr M. ALISON WILSON, Plymouth (Library).
Miss M. J. WOOD, London (Algae).
M. YOSHIDA, London (*Psammechinus* pigments).

Among the many other scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Dr Talbot H. Waterman (U.S.A.), Dr A. E. J. Went (Dublin), W. G. Morison (Sarawak), J. L. Reid, Jr. (U.S.A.), Prof. J. Tokida (Japan), Dr R. W. Hiatt (Hawaii), Dr M. S. Gordon (U.S.A.), S. C. Otuka (Nigeria), J. Davids (Holland), B. V. Hamon (Australia), Dr W. B. Hartman (U.S.A.), Dr T. F. Goreau (U.S.A.), P. Gabel (U.S.A.), Dr Dorothy C. Saunders (U.S.A.), Dr A. E. Parr (U.S.A.), Dr L. Provasoli (U.S.A.), G. Michanek (Sweden), Prof. Ki-Chul Choi (S. Korea), Prof. B. B. Benson (U.S.A.), Prof. N. W. Rakestraw (U.S.A.), F. B. Pithavadian (Madras), W. B. Bailey (Canada), Dr K. B. Markman (Sweden), Miss T. Vucetic (Yugoslavia), Prof. S. Motoda (Japan), Dr B. A. Ketchum (U.S.A.), Prof. Melom J. Cohen (U.S.A.).

About 120 delegates of the XVth International Congress of Zoology took the opportunity of visiting the Plymouth laboratory during their stay in this country. These included a party of thirty-three Russian scientists. Some of the delegates spent a day or so in the laboratory, and among these were: Dr H. L. Sanders (Woods Hole), Dr W. Trager (New York), Dr and Mrs K. Lagerspetz (Finland), Prof. E. Witschi (Iowa), Dr A. Bourdillon (Marseilles), Prof. Jean A. Lynsdale (Burma), Prof. and Mrs R. Defretin (Lille), Prof. and Mrs F. Bernard (Algiers), Dr and Mrs V. L. Loosanoff (Connecticut), Prof. and Mrs I. McT. Cowan (Vancouver), Prof. Erwin Kamptner (Vienna), Dr Lucia Rossi (Turin), Dr H. Stieve (Hamburg), Prof. and Mrs Carl Hubbs (La Jolla), Prof. Marie Gontcharoff (Sète), Dr H. G. Godlewski (Poland), Prof. J. H. Day (Cape Town), Prof. and Mrs R. Buchsbaum (Pittsburg), Dr O. Wetzel (Germany), Prof. Denzaburo Miyadi (Seto, Japan), Prof. P. Sawaya (São Paulo, Brazil).

The West German research vessel 'Anton Dohrn' visited Plymouth on 21 and 22 January 1959. The Captain, scientists and technical assistants were entertained at the Laboratory, including Dr G. Hempel, Dr E. Rogalla, Dr W. Gunkel and Dr H. J. Münzing.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty-one students from the following Universities: Oxford, Cambridge, St Andrews, Glasgow, Durham, London, Sheffield, Reading, Exeter, Hull, Swansea, Aberystwyth and Regent Street Polytechnic.

Also during the Easter Vacation Dr R. J. Jones and Dr C. T. Prime brought a party of twenty-one boys from Whitgift School, and Mr J. Peirson brought four boys from Rugby School.

Two University Courses were held at the Plymouth laboratory in September; a course in marine biology for ten students supervised by Miss J. E. Rigby and Miss S. Rogers of Queen Elizabeth College, and a course in marine botany for eighteen students supervised by Dr Margaret Naylor of the University of Hull.

Scientific work of the Plymouth Laboratory Staff

Sea Water and Plankton

For a third of a century many and varied studies on sea water and plankton have been concentrated upon a single accessible shallow-water station in the English Channel (E 1), supplemented by occasional more extensive excursions. This concentration of effort has been richly rewarded. A similar approach is appropriate in deep-water oceanography; a standard station has, therefore, been selected in a position, 46° 31′ N., 8° 00′ W., already worked twice by the Danish Research vessel 'Dana' in 1922 and 1930. This is the nearest deep-water station to Plymouth, and it has best possible coverage by the Decca navigator system. Since 1952 it has been repeatedly worked from R.R.S. 'Discovery II' and R.V. 'Sarsia'. During 1958 the programme has been co-ordinated with the requirements of the International Geophysical Year. Three cruises on R.V. 'Sarsia' in March, April and September have been organized by Dr L. H. N. Cooper, together with work from R.R.S. 'Discovery II' in the late autumn. This station, with continued study, should eventually provide part of a framework not obtainable by other IGY ships widely dispersed over the ocean.

A picture of the very considerable fluctuations which take place in the deep ocean has already emerged. Water which is physically homogeneous is not always chemically so. The mean oxygen content of the deep water decreased from a maximum in 1922 through 1930 to 1952, but is now once more steadily approaching the high figure of 1922. The results bear on the vexed issue of the 'age' of deep oceanic water.

The main oceanic position is complemented by two further standard stations over the continental slope off La Chapelle Bank and one at the nearby break of slope. Only after seven years are the data from these stations enabling the very important processes of vertical and horizontal mixing, and the leat currents which occur only in the blanket of water over continental slopes, to be differentiated. It seems likely that mixing processes are trifling in the deep open ocean, and that the appearance of such at any depth is a consequence of mixing processes against slopes and horizontal displacements of the mixed waters created there.

For practical reasons the distribution of the rarer constituents of sea water has everywhere been studied largely in the shallow seas. The reservoir stocks in the deep ocean remain unknown because of the difficulty of getting uncontaminated samples. Thus, much attention is being given to the technique of deep sampling for trace constituents for analyses to be made at other laboratories. During cruises in 1958 a range of samples to the bottom (4700 m) at the standard station were obtained for vitamin B_{12} (Dr K. Daisley, Shinfield), cadmium (Dr J. P. Riley, Liverpool), copper (Mr F. A. J. Armstrong), uranium (Mr G. C. Milner, Harwell), and state of oxidation of uranium (Dr D. C. Stewart, Argonne National Laboratory, Chicago and Harwell). From the same hoists deep-water samples were collected for studies on deep-dwelling flagellates and allied organisms (Dr Mary Parke). Mr E. I. Butler has helped much on these cruises.

It is known in broad terms that the continental slope south-west of the British Isles has a very rugged topography, but the present experimental approach requires concentration of effort on a few selected small areas whose topography is accurately charted. With the assistance of Mr G. W. Battin the necessary close surveys have been started at the edge of La Chapelle Bank. Measurements of the current at the break of slope of the bottom water in this area have been initiated by Mr E. I. Butler.

Dr Cooper and Dr T. I. Shaw have published their theoretical studies on the state of iodine in sea water in *Nature, Lond.*, Vol. 180, p. 250, and Vol. 182, p. 251.

Mr F. A. J. Armstrong has continued his analyses for ammonia and total inorganic nitrogen in monthly samples from the International Hydrographic Station E 1. In July 1958 inorganic nitrogen fell to less than 5% of the winter maximum values, whereas phosphate fell only to about 15%, so that nitrogen seems to be the limiting micronutrient for plant growth. Nitrogen-phosphorus ratios were about 20:1 by atoms or 9·2:1 by weight; these are a little higher than those found (16–17:1 by atoms) in 1931 by Dr Cooper. Recently nitrogen has been a little higher and phosphate lower than in 1931; silicate values have been similar. Phosphate and silicate analyses for Station E 1 during 1956 have been published in Vol. 37, No. 2, of the *Journal*, and those for 1957, together with some nitrogen figures, have been described jointly with Mr E. I. Butler, in Vol. 38, No. 1, of the *Journal*.

In an attempt to define the accuracy and precision of phosphate determinations Mr Armstrong has investigated three of the more reliable molybdenum blue methods, two using stannous chloride and the other ascorbic acid as reductant. Eight or ten replicate analyses are run at a time, and if filtered sea water is used, and all absorbancies measured on the same spectrophotometer, all three methods are surprisingly precise. The three methods give, however, three different results with the same sample of sea water, with differences of more than 0·1 mg atom P/l. between two methods. Part of these differences may be put down to the effect of arsenic in the water, since the methods are subject to differing degrees of interference from this element. This may make it very difficult to compare phosphate analyses from different laboratories.

Reduction of dissolved oxygen by corrosion of the metal when sea water is held in brass hydrographic bottles has been measured. In an extreme case the loss of oxygen was 0·3 ml. O_2/l. in 1¾ h at 16° C, and a rough equivalence has been found between the amount of oxygen reduced and the quantities of copper and zinc dissolved. Coating the insides of bottles with a cold-curing epoxy resin (Araldite No. 820 RH) was found to reduce oxygen loss to negligible proportions, and all the hydrographic bottles have been so treated. After repeated use at sea this year the coating shows no sign of deterioration.

In order to obtain large samples of deep ocean water, for some investigations in which the Laboratory assisted Dr G. C. Milner and Dr D. G. Stewart of Harwell, Mr Armstrong has designed and tested a 5 l. sampling bottle in which the sample does not come into contact with metal. A set of these bottles, of welded polythene, with rustless steel frames and release mechanisms was made by the Engineering Services Division of A.E.R.E. Harwell. After some initial difficulties the bottles have worked well at sea, and Mr Armstrong has been able to use them for collection of water for analysis for copper, trying out a very simple method using bis-cyclohexanone oxalyl dihydrazone, for which no solvent extraction is necessary.

Mr Armstrong has visited the laboratory of the Ministry of Agriculture, Fisheries and Food at Lowestoft where, by courtesy of the Director and with instruction from the Staff, he recalibrated about eighty of the deep-sea reversing thermometers belonging to the Plymouth laboratory. Some of them appear to have altered markedly since they were first calibrated, and the new corrections have generally given more consistent agreement between pairs of thermometers when applied to the observations made in the last 2 or 3 years.

With the co-operation of Mr E. I. Butler monthly samples of sea water have been taken throughout the year, usually from R.V. 'Sula', at Stations L 2–L 6 and E 1 (surface to 70 m) for the examination of the nanoplankton by Dr Mary Parke and Miss I. M. Adams. From these routine samples very numerous temporary cultures have been set up and grown on a special apparatus designed and built by Mr F. G. C. Ryder. With Dr L. H. N. Cooper's co-operation additional sea-water samples, taken at a series of stations out over the continental shelf into oceanic water (from surface down to 4000 m) during the March, April and September cruises of R.V. 'Sarsia', have also been cultured and have given useful data for comparison with those obtained from the more inshore stations. In addition to the culturing of samples, quantitative and qualitative examinations have also been made of a series of samples for Mr R. I. Currie of the National Institute of Oceanography to give him information to use with his results obtained by pigment analysis and by the ^{14}C method.

From this study of the nanoplankton interesting information is being accumulated. It had been found that the very small 1–2 μ pigmented flagellates are very widely distributed and are at times extremely common. A number of these forms have now been isolated and studied, but because of their very small size it is extremely difficult to be certain to which class of algae they belong, and therefore co-operation is being given by Prof. Irene Manton of Leeds University in their examination with the electron microscope, and by Dr G. Y. Kennedy of Sheffield University in analysing their pigments. All the forms so far examined have been found to belong to the Chlorophyceae, including the very commonly occurring organism originally described as a member of the Chrysophyceae, *Chromulina pusilla* Butcher.

This study has also confirmed that members of the genus *Chrysochromulina* (Chrysophyceae) are extremely well represented in the nanoplankton and it has also shown that species of the genus *Pseudopedinella* (Chrysophyceae), both pigmented and unpigmented, are of very frequent occurrence. A number of different species of this genus, therefore, including the type species, have already been isolated for future study by Dr Parke and Miss Adams. Isolations and the culturing of the 'Phaeocystis' type colonies, occurring at all times of the year, sometimes in great abundance, are also being continued for the study of the different types of motile stages produced by them. Some time has also been spent on the isolation and culture of numerous clones of the very commonly occurring *Coccolithus huxleyi* (Lohm.) Kamptner for a study of its form range and life history. Isolations have been made at different depths from inshore samples and from samples from oceanic stations as two strains differing in size may be involved, the larger strain occurring in oceanic water. Prof. E. Kamptner of Vienna, at his own request, is examining all the clone cultures that have been isolated.

From the temporary cultures set up a number of other interesting new forms have been isolated for future study, including new species of the genus *Chrysochromulina* and other forms belonging to the Chrysophyceae which will extend our knowledge of the form range to be found among marine members of this class. For example, a find of great botanical interest was made when Miss Adams, who has been responsible for the isolation of all new nanoplankton forms, found an organism she recognized as a new type which, with the help of electron micrographs taken by Prof. Manton, Dr Parke has now identified as *Crystallolithus hyalinus* Gaarder & Markali recently described (1956) from preserved material as a new genus and species of the Coccolithophoraceae. Two strains of this organism, isolated from different localities, are now being grown successfully in culture. The study of this coccolith-bearing organism in the living state has shown that it is an extremely valuable find as its structure gives further evidence for classifying the Coccolithophoraceae in the class Chrysophyceae. Also, like species of the genus *Chrysochromulina*, it is phagotrophic and in addition to possessing two flagella (not observed in the preserved material when the organism was described), it has also an unmistakable haptonema similar to that possessed by the members of the genus *Chrysochromulina*, on the form range of which Dr Parke is continuing to work. A further paper on this genus, in collaboration with Prof. Irene Manton, has been published in Vol. 38, No. 1, of the *Journal*. In this paper a species very common in the English Channel is described, and for the first time records for distribution and seasonal occurrence at six stations are included. Unlike any of the previously known species this new form is characterized by a very long haptonema capable of attaching at any point along its length and a two-layered covering of small scales, the scales of the two layers being of different size and pattern. The anatomy of the haptonema of this species is similar to that described for *C. chiton*, except that the number of fibres in the ring is six and not seven.

The collection of unialgal cultures of marine phytoplankton organisms continues to be in great demand by research workers in this country and abroad to whom over 200 cultures have been distributed.

Dr F. S. Russell has made a study of the biology of the deep-sea scyphomedusa *Atolla*. It was hoped that regular observations over the year on size and maturity would give an indication of length of life. It was found, however, that although mature specimens occurred in two size ranges, small and large, both groups were present throughout the year. A detailed examination of the small mature specimens has now shown that they are specifically distinct from the large mature *Atolla wyvillei*. The new species has been named *A. parva* and was briefly described in *Nature, Lond.*, Vol. 181, p. 1811. A full account of these observations has been published in Vol. 38, No. 1, of the *Journal.*

Work by Mr G. M. Spooner on the planktonic Amphipoda Hyperiidea has been mainly devoted to *Cystosoma*, a peculiar deep-water genus of large transparent forms, much of whose biology has hitherto remained obscure. Thanks to a collection made by Dr E. J. Denton on a cruise to the Bay of Biscay in R.V. 'Sarsia', by which the number of recorded adults of one of the species was doubled, the breeding cycle could be followed. The numerous small eggs are carried in an invaginated brood pouch in which they develop, as it proves, into a peculiar larval form unlike anything previously known in Amphipoda, resembling in appearance the microniscid larvae of Isopoda. A considerable change in structure must occur between this stage and the 'Physosoma' stage which precedes the final adult form.

Dr A. J. Southward has been examining rapid methods for sampling the plankton 'indicator' species in the Channel, and has made several surveys with a Lowestoft type Gulf III high-speed plankton sampler. This instrument makes good catches of euphausids and chaetognaths, and most of the Western Channel can be sampled in a few days. Results are still being analysed, but already they are producing a picture of the changing conditions of offshore Channel waters in relation to those in the vicinity of Plymouth. In the first half of 1958 *Sagitta elegans* was dominant throughout the Western Channel, and off the North Cornish coast, but appeared in comparatively large numbers at the Plymouth stations (Eddystone and E1) only in March.

In agreement with this, Mr P. G. Corbin has observed no major change in the low level of macro-plankton associated with the prevailing *Sagitta setosa* in the 2 m. stramin ring trawl collections during 1958.

Macro-Fauna and Flora

During the year many additions have been made to the herbarium and collection of preserved marine algae.

In 1939 Dr D. P. Wilson began a study of the developments of three species of the polychaete family Magelonidae, but the war made it impossible to continue. Of the three species one was the common *Magelona papillicornis* F. Müller, one a new and still undescribed species from Salcombe, whilst the third, abundant in the Rame Mud, was provisionally identified by him as *M. cincta* Ehlers. Recent searches of the Rame Mud and other likely muddy-sandy areas have shown that this latter species is no longer abundant, and only a few fragments of the worm have this year been obtained. A close study of these and of specimens collected in 1939 has established that it is a previously unrecognized species differing from *M. cincta* in several major features. Dr Wilson has described the worm and named it *M. alleni* after the late Dr E. J. Allen. He has also partially redescribed and refigured *M. cincta* Ehlers from the type specimen (kindly loaned by the Zoologisches Museum, Berlin) and from specimens recently collected on South African coasts by Prof. J. H. Day. From records by several workers and from specimens loaned by the British Museum (Natural History) it has appeared that *M. alleni* is widely distributed in the north-eastern Atlantic, while *M. cincta* is known only from southern and south-eastern coasts of Africa influenced by the warm Mozambique and Agulhas Current. A paper on this work has been published in Vol. 37, No. 3, of the *Journal*.

Dr Wilson has also published in Vol. 37, No. 2, of the *Journal* a third series of notes on observations made in the aquarium. In this series an account is given of the breeding behaviour of the Black Sea-bream, *Spondyliosoma cantharus* (Gmelin), of which no adequate description existed. Of even more interest are the observations on the previously unknown sexual display of the male Cuckoo Wrasse, *Labrus ossifagus* L., noteworthy for the remarkable white patch which appears on its head and shoulders during periods of intense sexual excitement, and which is not visible at other times.

Populations of the isopod *Jaera albifrons* (sens. lat.) have been examined by Mr G. M. Spooner in the Plymouth area, and elsewhere, to investigate the distribution of the so-called microspecies, which depend on differences in the secondary sexual development of certain limbs of the male. The animals are normally collected from the under side of stones, but *J. a. praehirsuta* typically confines itself to fucoid weeds. Investigation is handicapped because the females are apparently indistinguishable, and there is a low sex-ratio of males, because of the trivalent sex chromosome. Out of 2999 animals examined only 739 were male—a ratio not significantly different from that of 1 in 4 expected on theoretical grounds. In the Plymouth area *J. a. albifrons*, *J. a. forsmani*, *J. a. ischiosetosa* and *J. a. praehirsuta* are all common. There is wide over-lapping between these four, but only a single hybrid individual has been recognized. The 'microspecies' have rather different average optima, in the field—apart from the special substratum preference of *praehirsuta*—but they do not occupy clear zones on the shore as has been emphatically claimed on the Continent. The distribution of the very different *J. nordmanni* (now confirmed from Plymouth for the first time since the days of Spence Bate) has also been studied. It is confined to sites of much reduced salinity.

Mr Spooner has now found that the Eddystone shell gravel (depth 25–28 fathoms) is rich in the smaller species of malacostracan crustaceans, when appropriate means are taken to isolate the microfauna from samples of raw gravel. Out of thirty species so far seen, thirteen are new to the Plymouth fauna, some being hitherto undescribed. Among these are members of the isopod genus *Microcharon* and the amphipod family Bogidiellidae, both typical underground 'interstitial' groups, of which the majority of the known species (all small, blind, colourless) live in freshwater habitats. Others are known from intertidal sands usually affected by land drainage. These are the first true offshore records from anywhere. *Microcharon harrisi* has been described in Vol. 38, No. 1, of the *Journal*. The bogidiellid, represented at present by eleven damaged specimens, is being assigned to a new genus. These finds distinctly strengthen the idea, already suggested by the distribution of the aberrant amphipod *Ingolfiella*, that there exists an 'underworld' faunal element, spread universally through the earth's surface strata, irrespective of whether this is covered by sea, fresh water, or land, and independent of these elements for effective distribution.

The new species of Goby discovered by Mr G. R. Forster, while diving with aqualung equipment off the open coast-line, where rough reefs and cliffs form the shore, was described by Mr P. G. Corbin in *Nature, Lond.*, Vol. 181, p. 1659, and named *Gobius forsteri*.

Since Leach's (1815) original description of the swimming crab, *Portunus marmoreus*, it has remained a matter of difficulty to distinguish it from the closely related *P. holsatus* Fabricius and there has been much discussion about these two species in the literature, some authors considering them to be only variants of the same species. Mr Corbin has recently found a difference in the setation on the carpus and propus of the cheliped which readily separates the two species: it is a clear-cut difference and not one of superfine comparison.

Mr N. A. Holme has continued working through grab samples taken in previous years, and has completed a series taken in and around Quiberon Bay in 1955. Deposits in the bay are mainly of mud or muddy sand with a rich polychaete fauna, and are characterized by such molluscs as *Turritella communis*, *Thyasira flexuosa*, *Lucina spinifera* and *Dentalium* spp. The channel inside Belle Ile is scoured by currents, and the bottom is mainly of stones or gravel. *Amphioxus lanceolatus* was found at one or two stations, and the ophiuroid *Ophiopsila annulosa* was also recorded. Sorting of the sievings has been speeded up with no apparent loss of accuracy by using the flotation technique recently described by Birkett. Trichlorethylene was used, however, as it is less toxic than carbon tetrachloride.

A record of specimens of *Saxicavella jeffreysi* Winckworth taken off Plymouth has been published in the *Journal of Conchology*. Although reported by Jeffreys from among trawl refuse from Plymouth, this species had not since been recorded from the area.

Further measurements of populations of *Venerupis rhomboides* have been made in order to determine if the form *sarniensis* described by Turton is in fact a separate species. It now seems clear that the proportions of the shell are directly related to depth of water, the broader and stouter form (*sarniensis*) occurring on the shore, while the more slender typical form occurs in 20–30 fathoms. It is possible to trace a gradation in shell proportions with increasing depth, and this has been confirmed for other populations outside the Plymouth area.

Mr G. R. Forster has continued his underwater studies on the rock fauna in the vicinity of Plymouth, for which the selection of suitable positions for diving has been made much easier by the provision of a small visual-type echo-sounder. During the summer many dives have been made to try to assess the effect which the population of large spiny sea-urchins, *Echinus esculentus* L., have on the sessile animals and algae.

The numbers of *Echinus* have been estimated quantitatively by counting all the specimens seen while swimming with a 2½ m. rod along a 50 m rope which has been laid out in a straight line along the sea bottom. Up to the present 598 *Echinus* have been counted; the total area covered being just over 3300 m^2. This gives a ratio of one *Echinus* to slightly more than 5·5 m^2 of rock surface.

The rate of browsing has been studied by placing six *Echinus* under a wire frame 1 m^2 in area covered with large mesh nylon netting. Two of these frames were arranged on the sea bottom in the natural habitat of the *Echinus*. After a few weeks the rock surface on which the *Echinus* had been able to browse was carefully observed and compared with that under the second frame which contained no *Echinus*. Three such tests have been made during the summer, two were unsuccessful owing to the frames being moved or disturbed by gales. In the successful test in September, however, in rather less than four weeks the rock surface appeared to have been almost completely swept clean of its thin covering of Bryozoa, small barnacles, encrusting ascidians, besides some brown and red algae.

Dr A. J. Southward has continued observations on the distribution and abundance of barnacles and other shore animals. No marked changes have occurred in the Channel for the past year. Near Plymouth, the immigrant barnacle *Elminius*, which has been very common in the estuaries for several years, is now present on the wave-beaten open coast and is increasing slowly in abundance. In collaboration with Dr D. J. Crisp, of the Marine Biology Station, Anglesey, Dr Southward spent seven weeks in Ireland re-investigating in detail the distribution of the shore fauna and flora. Among other things it was found that the abundance of the northern barnacle *Balanus balanoides* had increased considerably in the south and west compared with 1952, while *Elminius* is established at several centres on the east and south coasts. Dr Southward is continuing work on the use of electronic flash cinematography for the study of barnacle behaviour. Some preliminary results were incorporated in a film on barnacle behaviour shown at the International Zoological Congress in London in July, as part of a joint contribution with Dr Crisp (*Proc. XVth Int. Congr. Zool.*, Sect. III, paper 5).

Dr A. J. Southward and Dr Eve Southward are studying the distribution and systematics of the Pogonophora of the Atlantic. The discovery that these interesting relatives of the chordates are common on the continental slope is a rewarding result of continued prosecution of deep water dredging, and owes a great deal to the perseverance of Captain C. A. Hoodless and the crew of R.V. ‘Sarsia’. Preliminary reports on the Pogonophora have been published in *Nature, Lond.*, Vol. 181, p. 1607, and Vol. 182, p. 272; and two new species are described in Vol. 37, No. 3, of the *Journal*. A note on the systematics and behaviour of two deep-sea barnacles, *Hexalasma* and *Verruca*, was also published in the same issue of the *Journal*.

Physiology of Marine Organisms

Dr T. I. Shaw has prepared an account of his experiments upon ^{131}I uptake by the sea weed *Laminaria digitata*, and this has been accepted for publication in the *Proceedings of the Royal Society* B. Currently he has investigated the state of iodine in the tissues of the weed. Chemical analyses, based on precipitations with silver nitrate, have indicated that some three-quarters of the iodine taken into the tissues is retained there as inorganic iodide. Preliminary experiments using chromatography upon the unhydrolysed weed tissues have both confirmed this finding and have also shown that the remaining iodine, being immobile upon the chromatographs, can be neither mono-, nor di-iodotyrosine.

Further studies upon the intermediate compounds of carbohydrate metabolism have shown that citric acid is increased in concentration in the tissues of the weed during the uptake of iodide.

In collaboration with Prof. A. L. Hodgkin, F.R.S., of Cambridge, and Dr P. C. Caldwell, Alan Johnston, Lawrence and Mosely Research Fellow of the Royal Society, Dr Shaw is also investigating the influence of phosphate esters upon the active transport of sodium and potassium across the membrane of the giant nerve fibres of *Loligo*. Factors influencing the active transport of sodium and potassium are of the widest general interest in biology, and it is important to try and obtain an understanding of the mechanisms involved. A great deal of work in the field has already been carried out at Plymouth by Dr R. D. Keynes of Cambridge and Dr Caldwell, and the present investigations arise directly from their earlier observations. This season’s work has principally centred upon the effects of injecting arginine phosphate into the fibres poisoned with either dinitrophenol or cyanide. It has been shown that the effects upon the sodium efflux are markedly dependent both upon the state of poisoning and upon the presence or absence of potassium in the external sea water. For example, arginine phosphate reduces the sodium efflux from fibres poisoned with dinitrophenol (applied in artificial sea water containing bicarbonate) provided that potassium is absent from the external medium, but markedly increases the sodium efflux from fibres poisoned with cyanide. Experiments are also being carried out to determine the time course of changes in the sodium efflux induced by altering the external potassium concentration.

Most work on luminescent animals has hitherto been carried out on large multicellular species (Metazoa), but there are certain advantages in studying protozoans. *Noctiluca* is especially suitable, since the cells are large enough to be seen and handled easily. An experimental study of *Noctiluca* has been carried out by Dr J. A. C. Nicol, both in the laboratory and at sea. This species sometimes occurs in dense patches, in concentration sufficient to produce 'red tides', and producing bright phosphorescence at night. Although the light from the aggregation of cells appears like a bright glow or wave, the light from each cell is a quick flash, some 0·1 sec in duration. By controlled electrical and mechanical stimulation of single cells, it has been possible to show that luminescence is subject to fatigue. The cells recover in about a minute; consecutive flashes may become brighter (facilitation). The luminescent responses are much like those observed in higher animals possessing nervous systems. Presumably, a similar system of excitation is involved: some form of surface action takes place when the cell is excited, leading to a controlled release of a brief photogenic reaction. In higher animals the excitatory stimulus is normally nervous.

A further study has also been made by Dr Nicol of sea pens collected by R.V. 'Sarsia' in the Bay of Biscay. Earlier studies have shown how the nervous system (a nerve net) controls the transmission of light waves across these animals. In the present investigation isolated zooids or polyps have been studied. These emit brief flashes, lasting about $\frac{1}{5}$th of a sec, and having an intensity of $0{\cdot}1 \times 10^{-9}$ to 63×10^{-9} μJ/cm^2 receptor surface at 1 cm distance. The additive effect of the flashes of separate zooids produces waves lasting about 1 sec at each locus, and progressing over the animal at a velocity of 6 cm/sec. The intensity of light emitted by the whole animal ranges from $0{\cdot}8 \times 10^{-6}$ to 7×10^{-6} μJ/cm^2 receptor surface at 1 cm distance. Luminescence in sea pens is accompanied by contraction and withdrawal. In its normal habitat, the animal, when disturbed, would give a flash visible for several metres, and then contract and pull itself down into the mud, in which it is anchored. It would be desirable to have some information about how quick weak flashes of this kind affect other active animals. The results of this research have appeared in Vol. 37, No. 3, of the *Journal*.

Dr Nicol has also published in Vol. 37, No. 3, of the *Journal* the results of his work on the luminescence of animals in a number of different groups, some of which was outlined in last year's report. In this paper observations are also given on the reflecting properties of red prawns and black fish. It was shown that the absorption characteristics are such as to provide minimum reflection of blue light prevailing in the deep sea and of luminescent light, thus having adaptive significance. Estimates have also been made of the distances at which the luminescence of animals can be seen in the sea ranging from below 10 to about 100 m according to the brightness of the organism.

Dr E. J. Denton has continued his experiments on the vision of fishes. The layers of retinae other than the receptor layer have been shown to have a very small absorption of light and, since this absorption varies little with wavelength, it could only account for very small changes in spectral sensitivity. The observations on the polarization of the fluorescence of the vitamin A in the bleached retinal rods which was described last year have been confirmed on other species. An account of this work consisting of observations on the retinae of various animals chosen so as to give examples of golden, red and purple retinae has been published in the *Proceedings of the Royal Society* B.

Experiments have been made to show that the retinal rods, because of their high refractive index, channel light coming to them. This allows the layer of receptors, although often relatively very thick, to act effectively as a 'thin emulsion' and thus to allow accurate vision. It has been shown that to give the best retinal image the lens must form its image on the internal end of the receptors. This has been demonstrated for several coastal fish, the frog and for several species of deep-sea fish. The retinae of deep-sea fish are extremely favourable for this experiment because the rods are very long and the other layers of retina very thin.

The experiments undertaken by Dr Denton in collaboration with Dr D. B. Carlisle on the change in visual pigments during the metamorphosis from the yellow to the silver eel have been completed by histological measurements. These showed that although the area of retina increases about fourfold on metamorphosis the number of receptors remains constant. The retinal rods about double, however, in diameter, and this accounts for the fact that the optical density of pigment in the retina remains high. A deep-sea eel, *Synaphobranchus* sp., has been shown to have a golden retinal pigment almost identical with those of the silver fresh-water eel and the conger eel. The results of this work have been published in Vol. 38, No. 1, of the *Journal*.

Further observations have been made, with the assistance of Mr F. J. Warren, on the spectral absorption of light by the crystalline lenses and corneas of fish and squid. In the freshwater perch a curious system exists in which the selective absorption of light on its way to the retinae takes place in two stages. The crystalline lens absorbs all the ultra-violet light below 400 mμ whilst the cornea, which is transparent in the ultra-violet, absorbs the blue region of the spectrum. In other species all this absorption is done by the lens alone.

Dr Denton's work with Mr N. B. Marshall, of the British Museum (Natural History), on the buoyancy of deep-sea fish without a swimbladder has been completed and has been published in Vol. 37, No. 3, of the *Journal*.

Dr Denton and Dr Shaw have studied the physiology of a very notable adaptation for buoyancy found in the Cranchid squid. Dr J. Gilpin Brown, a visiting research worker, is collaborating with them and is studying the histology and anatomy of these squid with particular reference to this problem. These squid are in buoyancy equilibrium with sea water. They achieve this equilibrium by balancing their heavier parts by a very large volume of coelomic fluid which is less dense than sea water, its density being 1·010. This coelomic fluid accounts for about two-thirds of the mass of squid. The fluid is almost isotonic with sea water showing that buoyancy is not achieved by simple exclusion of solutes. The low density results from replacing most of the cation of sea water by ammonium ions which exist in the fluid in a concentration of almost 500 mM. The anion content is almost exclusively chloride. The fluid is very acid, pH 5·2, and this is doubtless connected with the retention of ammonia in the coelom. In design these squids are strikingly similar to the bathyscaphe, relatively recently designed by Professor Piccard, in that they balance their useful but dense parts by a large flotation chamber containing a fluid of lower specific gravity than sea water. A brief account of this work has been published in *Nature, Lond.*

An investigation begun last year by Dr D. B. Carlisle, in collaboration with Dr Peter Karlson of Munich, into the chemistry of crustacean hormones is bearing some fruit. A sample of chromactivating substances of which 10^{-16} g is enough to produce a response in an individual prawn has been prepared. This substance is, however, not yet pure, and work is in progress to obtain a much purer sample of this material for chemical analysis. It has been shown that the chromactivating substance found in impure samples of ecdyson obtained from silk worms, *Bombyx mori*, is not ecdyson itself but some impurity. Insect ecdyson has itself no action on colour change of prawns but stimulates their moulting activity. It is not, however, as potent as the similar material obtained from *Crangon*. Conversely, the material isolated from *Crangon* is less active on insects than insect ecdyson. Dr Carlisle has published an account in *Nature, Lond.*, Vol. 182, pp. 32–4, on the inactive precursor of a chromactivating substance of the sinus gland of *Leander* and on the ways in which this inactive substance may be converted into the active hormone.

Dr Carlisle has investigated further the endocrine basis of sex reversal in *Pandalus*. He has found evidence that the vas deferens gland participates in the control of this phenomenon. His account of neurosecretory transport in the pituitary stalk of *Lophius piscatorius* has been published in *Zweites Internationales Symposium über Neurosekretion* (Springer–Verlag, Berlin), 1958.

Dr Carlisle is now Recorder of the Protochordate Section of the *Zoological Record*, and for this section the Record for 1955 and 1956 has been published in the course of the year.

Dr Carlisle's work with Mr L. G. Hummerstone on niobium in ascidians and in the sea has continued and two papers on the subject have appeared in *Nature, Lond.*, Vol. 181, pp. 933 and 102–3.

In collaboration with Dr P. C. J. Brunet of Oxford, Dr Carlisle has been investigating the exuvia, cuticle or tube of a number of species of marine animals to determine their composition. The most worthwhile result to date is that the tube of four species of Pogonophora consists primarily of chitin and protein, contrary to the statement of A. V. Ivanov who reports that these tubes consist of cellulose or tunicin. A preliminary account of this work has appeared in *Nature, Lond.*, Vol. 182, p. 1689, and it is hoped to publish a fuller account in the *Journal.*

While Dr Carlisle was at the Kristineberg Laboratory in Sweden, pursuing his investigations of *Pandalus*, he observed moulting to take place in *Priapulus* and found that the exuvia of this species consists of chitin and tanned protein while the new cuticle of the post-moult individual contains only protein, the chitin portion appearing as the cuticle hardens and thickens. An account of this investigation is in the press in *Arkiv för Zoologi, Stockholm.*

Dr Carlisle has continued employing diving equipment to study the biology of crabs, prawns and ascidians in their natural habitats.

Dr E. D. S. Corner has continued his studies of the poisoning of *Maia squinado* by mercury. ^{203}Hg-labelled $HgCl_2$ and n-$C_5H_{11}HgCl$ have been used in order to compare the distributions of mercury compounds of different lipoid solubilities throughout the test animals, and it has been found that whereas the organomercury compound is readily assimilated by various internal organs, notably the antennary glands, most of the inorganic compound remains attached to proteins in the blood. Paper chromatography has been used in attempts to identify amino acids present in the blood and urine of *Maia*, and although no marked differences have been found in the relative quantities of amino acids in the body fluids of poisoned and control animals, further quantitative experiments have shown that *Maia* treated with mercury poisons excretes greatly increased amounts of urinary amino-N, usually without any corresponding increase in the blood level. It has also been found that quantities of $HgCl_2$ that cause a marked increase in urinary amino-N have little effect on the excretion of total sulphate. These findings are consistent with the view that mercury compounds impair the function of the antennary glands by inhibiting the process by which amino acids are re-absorbed from the urinary filtrate into the blood. A paper describing these results has been accepted for publication in *Biochemical Pharmacology.*

In collaboration with Dr R. D. Bulbrook, of the Royal College of Surgeons, an attempt is being made by Dr Corner to extract from marine invertebrates an enzyme catalyzing the hydrolysis of androsterone sulphate. This sulphatase would be of considerable use in analyses of androsterone in conjugated form. So far the digestive glands of *Maia squinado, Cancer pagurus* and *Buccinum* have been examined, but the preparations obtained from these organs possessed only small activity. It is hoped that continued search will eventually disclose a source of suitably active material.

Dr Corner and Mr B. W. P. Sparrow (International Paints Research Laboratory, Newton Ferrers) have extended their studies of the effects of toxic agents on marine organisms to include experiments with the intertidal red alga *Plumaria elegans*. In collaboration with Mr A. D. Boney (Plymouth Technical College) a method has been devised for comparing the effects of heavy metals and other more specific respiratory inhibitors on the growth and viability of *Plumaria* sporelings. In general, it has been found that the test organisms are very resistant to poisoning by heavy metals and the correlation between lipoid solubilities and toxicities, especially within the homologous series of primary *n*-alkylmercuric chlorides, is less exact than that observed in similar experiments using crustaceans. In a complementary investigation, carried out primarily by Mr Boney, a comparison has been made of the abilities of various species of red algae to withstand harsh environmental conditions and the resistances of these plants to poisoning by certain heavy metals. Results obtained so far have been encouraging in that ecological and toxicological data show a close correlation. An account of this work is being published in *Biochemical Pharmacology*.

During his year at Plymouth, Mr B. R. Jewell was engaged in investigations of the mechanical and thermal properties of the anterior byssal retractor muscle (ABRM) of *Mytilus*. Initially, a good deal of time was devoted to making improvements in the apparatus and methods that are used in experiments on the ABRM; these included developing a method of isolating intact preparations of the muscle (demonstrated to the Physiological Society in March, 1958) and designing a new type of multi-electrode assembly, which was constructed by Mr F. G. C. Ryder; the improvement in the standard of the experimental work amply justified the overall expenditure of time and effort.

The experiments revealed striking differences in the mechanical state of the ABRM during phasic and tonic responses. During the former, the ability of the muscle to do work and to re-develop tension after it has been allowed to shorten is much greater than it is during the latter. Such findings cannot easily be explained by the 'tetanus' hypothesis, for they suggest that the phasic and tonic responses are not based on the same fundamental unit, the muscle twitch.

Some preliminary studies of the thermal properties of the ABRM were made with Mr J. V. Howarth, in Prof. A. V. Hill's laboratory at University College, London. It was shown that, in the presence of oxygen, the tonic response is accompanied by a sustained increase in the heat production of the muscle. Other results suggested that, in the absence of oxygen, a reversible state may be reached in which the muscle can maintain tension without the expenditure of any energy.

LIBRARY

During the year the librarian, Miss L. M. Serpell, visited a number of libraries at other marine laboratories and institutions. Visits were also made to the library of the Plymouth laboratory by other librarians. Such meetings contribute towards closer liaison between the libraries.

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

PUBLISHED MEMOIRS

Vol. 37, No. 2, of the *Journal* was published in June, Vol. 37, No. 3, in October 1958, and Vol. 38, No. 1 in February 1959.

The following papers, the outcome of work done at the Plymouth laboratory, have been published elsewhere than in the *Journal* of the Association:

ABBOTT, B. C., AUBERT, X. & FESSARD, A., 1958. La production de chaleur associée à la décharge du tissu électrique de la Torpille. *J. Physiol., Paris*, Tome 50, pp. 99–102.

ABBOTT, B. C. & LOWY, J., 1958. Contraction in molluscan smooth muscle. *J. Physiol.* Vol. 141, pp. 385–97.

ABBOTT, B. C. & LOWY, J., 1958. Mechanical properties of *Helix* and *Mytilus* muscle. *J. Physiol.*, Vol. 141, pp. 398–407.

ALLEN, J. A., 1958. On the basic form and adaptations to habitat in the Lucinacea (Eulamellibranchia). *Phil. Trans.*, B, Vol. 241, pp. 421–84.

ATKINS, D., 1958. British Pea-crabs (*Pinnotheres*). *Nature, Lond.*, Vol. 181, p. 1087.

ATKINS, D., 1958. A new species and genus of Kraussinidae (Brachiopoda) with a note on feeding. *Proc. zool. Soc. Lond.*, Vol. 131, pp. 559–81.

ATKINS, W. R. G., 1957. The direct estimation of ammonia in sea water, with notes on nitrate, copper, zinc, and sugars. *J. Cons. Int. Expl. Mer*, Vol. 22, pp. 271–77.

ATKINS, W. R. G. & POOLE, H. H., 1958. Cube photometer measurements of the angular distribution of submarine daylight and the total submarine illumination. *J. Cons. Int. Expl. Mer*, Vol. 23, pp. 327–36.

BLASCHKO, H. & HOPE, D. B., 1957. Observations on the distribution of amine oxidase in invertebrates. *Arch. Biochem. Biophys.*, Vol. 69, pp. 10–15.

BODEN, B. P. & KAMPA, E. M., 1958. Lumière, bioluminescence et migrations de la couche diffusante profonde en Méditerranée occidentale. *Vie et Milieu* (Bull. Lab. Arago), Tome IX, 10 pp.

BONE, Q., 1958. Synaptic relations in the atrial nervous system of *Amphioxus*. *Quart. J. micr. Sci.*, Vol. 99., pp. 243–61.

BRUNET, P. C. J. & CARLISLE, D. B., 1958. Chitin in Pogonophora. *Nature, Lond.*, Vol. 182, p. 1689.

CALDWELL, P. C., 1958. Studies on the internal pH of large muscle and nerve fibres. *J. Physiol.*, Vol. 142, pp. 22–62.

CAMBRIDGE, G. W., 1958. Responses of the anterior retractor muscle of the byssus of *Mytilus edulis*. *Nature, Lond.*, Vol. 182, p. 35.

CALDWELL, P. C., 1959. Quelques relations entre le métabolisme et les transports actifs d'ions dans l'axone géant du Calmar. *The Method of Isotopic Tracers applied to the Study of Active Ion Transport* (1er Colloque de Biologie de Saclay), pp. 88–94. London.

CARLISLE, D. B., 1958. Niobium in ascidians. *Nature, Lond.*, Vol. 181, p. 933.

CARLISLE, D. B., 1958. A crustacean chromactivator. *Nature, Lond.*, Vol. 182, pp. 33–4.

CARLISLE, D. B., 1958. Voyages of H.M.S. 'Beagle'. *Sci. News*, Vol. 50, pp. 7–24.

Carlisle, D. B., 1958. Neurosecretory transport in the pituitary stalk of *Lophius piscatorius*. *Zweites internat. Symposium Neurosecretion, Lund*, 1957, pp. 18–19. Berlin: Springer-Verlag.

Carlisle, D. B. & Denton, E. J., 1957. A change in visual pigments in the life of the fresh-water eel. Proc. physiol. Soc., 27–28 September 1957. *J. Physiol.*, Vol. 139, 8*P*.

Carlisle, D. B. & Hummerstone, L. G., 1958. Niobium in sea water. *Nature, Lond.*, Vol. 181, pp. 1002–3.

Carlisle, D. B. & Jenkin, P. M., 1959. Terminology of hormones. *Nature, Lond.*, Vol. 183, pp. 336–37.

Carlisle, D. B. & Knowles, F. G. W., 1959. *Endocrine Control in Crustaceans.* Cambridge: University Press, 120 pp.

Cooper, L. H. N., 1958. Consumption of nutrient salts in the English Channel as a means of measuring production. *Rapp. Cons. Explor. Mer*, Vol. 144, pp. 35–7.

Corbin, P. G., 1958. A new British fish (*Gobius forsteri*). *Nature, Lond.*, Vol. 181, p. 1659.

Denton, E. J., 1957. Light absorption by the intact retina. *Paper 5, Symposium on Visual Problems of Colour held at the Nat. Phys. Lab. Teddington, September* 1957. 13 pp.

Denton, E. J., 1959. The contributions of the orientated photosensitive and other molecules to the absorption of whole retina. *Proc. roy. Soc.* B, Vol. 150, pp. 78–94.

Denton, E. J., Shaw, T. I. & Gilpin-Brown, J. B., 1958. Bathyscaphoid squid. *Nature, Lond.*, Vol. 182, pp. 1810–11.

Fisher, L. R. & Kon, S. K., 1959. Vitamin A in the invertebrates. *Biol. Rev.*, Vol. 34, 36 pp.

Green, J., 1958. *Dactylopusioides macrolabris* (Claus) (Copepoda: Harpacticoida) and its frond mining nauplius. *Proc. zool. Soc. Lond.*, Vol. 131, pp. 49–54.

Hawes, F. B., 1958. Preliminary observations on the settlement of the Actinula larva of the *Tubularia larynx* (Ellis & Solander). *Ann. Mag. Nat. Hist.*, Ser. 13, Vol. 1, pp. 147–55.

Hedley, R. H., 1958. A contribution to the biology and cytology of *Haliphysema* (Foraminifera). *Proc. zool. Soc. Lond.*, Vol. 130, pp. 569–76.

Hill, A. V. & Howarth, J. V., 1958. The initial heat production of stimulated nerve. *Proc. roy. Soc.*, B, Vol. 149, pp. 167–75.

Holme, N. A., 1959. *Saxicavella jeffreysi* Winckworth at Plymouth. *J. Conch.*, Vol. 24, p. 325.

Jewell, B. R., 1958. An improved method of preparing the *Mytilus* anterior byssal retractor muscle that avoids injuring the fibres. Proc. physiol. Soc., 21–22 March 1958. *J. Physiol.*, Vol. 142, 17*P*.

Jones, W. C., 1958. The effect of reversing the internal water-current on the spicule orientation in *Leucosolenia variabilis* and *L. complicata*. *Quart. J. micr. Sci.*, Vol. 99, pp. 263–78.

Murray, R. W., 1959. The response of the ampullae of Lorenzini to combined stimulation by temperature change and weak direct currents. *J. Physiol.*, Vol. 145, pp. 1–13.

Potts, W. T. W., 1958. The inorganic and amino acid composition of some lamellibranch muscles. *J. exp. Biol.*, Vol. 35, pp. 749–64.

Russell, F. S., 1958. Salt excretion in marine birds. *Nature, Lond.*, Vol. 182, p. 1755.

Russell, F. S., 1958. A new species of *Atolla*. *Nature, Lond.*, Vol. 181, pp. 1811–12.

SHAW, T. I. & COOPER, L. H. N., 1958. Oxidized iodine in sea water. *Nature, Lond.*, Vol. 182, pp. 251–52.

SOUTHWARD, A. J., 1958. The zonation of plants and animals on rocky sea shores. *Biol. Rev.*, Vol. 33, pp. 137–77.

SOUTHWARD, A. J., 1958. Abundance of Pogonophora. *Nature, Lond.*, Vol. 182. p. 272.

SOUTHWARD, A. J. & SOUTHWARD, E. C., 1958. Pogonophora from the Atlantic. *Nature, Lond.*, Vol. 181, p. 1607.

MEMBERSHIP OF THE ASSOCIATION

The total number of members on 31 March 1959 was 1035, being 41 more than on 31 March 1958; of these the number of life members was 121 and of annual members 914. The number of associate members is five, Mr G. M. Graham, C.M.G., O.B.E., having been elected during the year.

GRANT FOR AQUARIUM RECONSTRUCTION

The Council wishes to record their grateful thanks for the following donation towards the cost of reconstructing the aquarium:

Messrs Pilkington Brothers Ltd.	£50

MORLEY NEALE STAFF FUND

Mr Morley H. Neale, C.B.E., a Vice-President of the Association, has made a generous gift of £1300 to be used by the Director of the Plymouth laboratory at his sole discretion for the benefit and pleasure of the Association's staff. £1000 of this is to be invested and the annual interest used for the purposes of the Fund.

FINANCE

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Private Income. The Council gratefully acknowledge the following generous grants received during the year:

Fishmongers' Company (£400), The Royal Society (£100), British Association (£50), Physiological Society (£50), The Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s.*), Leeds (£20), Durham (£10. 10*s.*), Manchester (£10. 10*s.*), Sheffield (£10. 10*s.*), Southampton (£15. 15*s.*), Reading (£10. 10*s.*), Nottingham (£10. 10*s.*), Hull (£10. 10*s.*), Exeter (£10. 10*s.*), Leicester (£10. 10*s.*), The Imperial College of Science and Technology (£10), Gonville and Caius College, Cambridge (£5), and the Zoological Society of London (£10. 10*s.*).

President, Vice-Presidents, Officers and Council:

The following is the list of those proposed by the Council for election for the year 1959–60:

President

Prof. A. V. Hill, C.H., O.B.E., Sc.D., LL.D., F.R.S.

Vice-Presidents

The Earl of Iveagh, K.G., C.B., C.M.G.
Sir Nicholas E. Waterhouse, K.B.E.
Col. Sir Edward T. Peel, K.B.E., D.S.O., M.C.
Vice-Admiral Sir John A. Edgell, K.B.E., C.B., F.R.S.
Sir Edward J. Salisbury, Kt., C.B.E., D.Sc., F.R.S.
A. T. A. Dobson, C.B., C.V.O., C.B.E.
Major E. G. Christie-Miller
Morley H. Neale, C.B.E.
The Earl of Verulam
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.
G. M. Graham, C.M.G., O.B.E.

COUNCIL

To retire in 1960

Prof. E. Baldwin, Ph.D.
C. H. Mortimer, Dr.phil., D.Sc., F.R.S.
S. Smith, Ph.D.
Prof. J. E. Smith, Sc.D., F.R.S.
H. G. Vevers, M.B.E., D.Phil.

To retire in 1961

H. A. Cole, D.Sc.
G. E. Fogg, Ph.D.
Prof. J. E. Harris, Ph.D., F.R.S.
C. E. Lucas, C.M.G., D.Sc.
Prof. C. M. Yonge, C.B.E., D.Sc., F.R.S.

To retire in 1962

J. N. Carruthers, D.Sc.
Prof. A. L. Hodgkin, F.R.S.
Prof. O. E. Lowenstein, D.Sc., F.R.S.
Prof. G. E. Newell, Ph.D.
Prof. W. F. Whittard, D.Sc., F.R.S.

Hon. Treasurer

Harrison S. Edwards, Westhumble Lacey, Near Dorking, Surrey

Secretary

F. S. Russell, C.B.E., D.S.C., D.F.C., LL.D., F.R.S.
The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

R. G. R. Wall (Ministry of Agriculture, Fisheries and Food)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Major E. G. Christie-Miller
  Harrison S. Edwards
Prof. Sir Alister Hardy, Kt., D.Sc., F.R.S. (Oxford University)
C. F. A. Pantin, Sc.D., F.R.S. (Cambridge University)
Edward Hindle, Sc.D., F.R.S. (British Association)
N. B. Marshall (Zoological Society)
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)

BALANCE SHEET 1958–9

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31 MARCH 1959

	£	£
Capital Reserve Account:		
As at 31 March 1958	174,054	
Add: Expenditure on fixed assets recovered	625	
	174,679	
Less: Transfer to surplus account being an amount equivalent to the depreciation provided on assets acquired out of Development Fund grants	3,674	171,005
Surplus Account:		
As at 31 March 1958	13,522	
Add: Transfer from Capital Reserve Account	3,674	
Transfer from 'Plymouth Marine Fauna' Fund	1,100	
Decrease in provision for diminution in value of General Fund investments	70	
	18,366	
Deduct: Excess of expenditure over income for the year ...	8,888	9,478
		180,483
Balances on Special Funds (see annexed statement)		5,461
Current Liabilities:		
Sundry creditors and accrued expenses	2,179	
Subscriptions and grants received in advance	327	2,506
		£188,450

Note: Capital commitments outstanding amount to £8,695 (1958 £1,200) of which approximately £7,550 (1958–nil) is recoverable

O. D. Hunt
F. S. Russell } *Members of the Council*

	£ Cost or Valuation	£ Depreciation	£
Fixed Assets:			
Boats and equipment:			
At cost:			
R.V. 'Sarsia'	137,761	6,539	131,222
M.F.V. 'Sula'	12,500	815	11,685
R.L. 'Gammarus'	200	20	180
	150,461	7,374	143,087
Laboratory apparatus, equipment and machinery: At cost	17,070	5,320	11,750
Library at valuation in 1941 plus additions as valued by the Director	23,850	—	23,850
	£191,381	£12,694	
			178,687
Investments at Market Value:			
General Fund (including Composition Fees) at book amount (Market value £1,370; last year £1,220)		1,751	
E. T. Browne Bequest Funds at cost (Market value £3,431; last year £3,086)		4,689	
		6,440	
Less: Provision for diminution in value of investments		1,639	4,801
Current Assets:			
Stocks on hand as valued by the Director		3,614	
Sundry debtors and prepayments		1,051	
Balances at bankers and cash in hand		297	4,962
			£188,450

Auditors' Report to the Members of the Marine Biological Association of the United Kingdom:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31 March 1959 and of its excess of expenditure over income for the year ended on that date.

We have obtained all the information and explanations which we considered necessary. In our opinion the Association has kept proper books and the said accounts which are in agreement with them and with the said information and explanations, give in the prescribed manner the information required by the Companies Act 1948.

Norwich Union House
2 St Andrew's Cross
Plymouth
14 *May* 1959

Price Waterhouse & Co.
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 MARCH 1959

	£	£
SALARIES (including additional for previous year) NATIONAL INSURANCE, SUPERANNUATION SCHEME CONTRIBUTIONS AND SUPPLEMENTARY PENSIONS		34,846
LABORATORY AND BOATS' CREWS' WAGES (including additional for previous year), NATIONAL INSURANCE, SUPERANNUATION SCHEME CONTRIBUTIONS, PENSIONS AND EMPLOYERS' LIABILITY INSURANCE		38,482
UPKEEP OF LIBRARY		670
SCIENTIFIC PUBLICATIONS, *less* SALES		1,907
UPKEEP OF LABORATORIES:		
Buildings and machinery	1,409	
Electricity, gas, coal and water	1,513	
Chemicals and apparatus	3,283	
Depreciation of laboratory apparatus, equipment and machinery	1,302	
Rents and insurance	401	
Travelling expenses	831	
Audit fee	149	
Stationery, postage, telephone and sundries	1,381	
Specimens	200	
Collecting expenses and upkeep of truck	245	
		10,714
MAINTENANCE AND OPERATION OF BOATS:		
Petrol, oil, paraffin, etc.	2,189	
Maintenance and repairs	6,882	
Depreciation	3,674	
Insurances	2,356	
Hire of Decca Navigator—R.V. 'Sarsia'	395	
		15,496
ENTERTAINMENT EXPENSES		148
		£102,263

	£	£	£
GRANTS AND TABLE RENTS:			
Ministry of Agriculture, Fisheries and Food—Grant from Development Fund		83,871	
Fishmongers' Company		400	
Miscellaneous (including Royal Society £100, British Association £50, Physiological Society £50, Cornwall Sea Fisheries Committee £10, Universities of London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s*., Leeds £20, Southampton £15. 15*s*., Durham £10. 10*s*., Exeter £10. 10*s*., Leicester £10. 10*s*., Manchester £10. 10*s*., Nottingham £10*s*. 10*s*., Hull £10. 10*s*., Reading £10. 10*s*., and Sheffield £10. 10*s*., Imperial College £10, Zoological Society of London £10. 10*s*., Ministry of Works £96, Imperial Chemical Industries Ltd., £52. 10*s*., International Paints Ltd. £39. 7*s*. 6*d*., Gonville and Caius College, Cambridge £5)		1,344	
			85,615
SUBSCRIPTIONS			876
SALES:			
Specimens		2,937	
Fish		622	
Nets, gear and hydrographical equipment	2,307		
Less: Cost of materials...	1,037		
		1,270	
			4,829
INCOME FROM INVESTMENTS			58
INTEREST ON BANK DEPOSITS, *less* CHARGES			154
AQUARIUM:			
Admission fees		2,072	
Sale of guides		40	
		2,112	
Less: Maintenance, printing and advertising		269	
			1,843
BALANCE being excess of expenditure over income for the year			8,888
			£102,263

MOVEMENTS ON SPECIAL FUNDS DURING THE YEAR TO 31 MARCH 1959

	E. T. Browne Bequest										
	Library £	Special Apparatus £	Scientific Publications £	Aquarium Reconstruction Fund £	Rockefeller Foundation Fund £	Library Extension and Dogfish House Fund £	'Plymouth Marine Fauna' Fund £	Reservoir and Sea Water Tanks Fund £	Algal Culture Fund £	Research Funds* £	TOTAL £
BALANCES AT 31 MARCH 1958 (after providing £1,496 for diminution in value of investments)	975	1,835	528	1,172	14	679	1,100	834	178	354	7,669
Add: Income during year											
Grants (including amounts paid direct to contractor by the Nuffield Foundation)	—	—	—	4,128	1,383	—	—	—	—	3,362	8,873
Income from investments	40	83	26	—	—	—	—	—	—	—	149
Bank deposit interest	—	—	—	39	—	18	—	36	2	—	95
Other income	—	—	69	—	—	—	—	—	—	—	69
Reduction in provision for diminution in value of investments	53	152	33	—	—	—	—	—	—	—	238
	1,068	2,070	656	5,339	1,397	697	1,100	870	180	3,716	17,093
Deduct: Expenditure during year	10	—	—	4,198	896	—	—	1,775	180	3,473	10,532
Transfer to General Fund	—	—	—	—	—	—	1,100	—	—	—	1,100
BALANCES AT 31 MARCH 1959	£1,058	£2,070	£656	£1,141	£501	£697	—	£(905)	—	£243	£5,461

* Including International Paints Ltd. Research Fellowship.